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APPLICANTS:	JUTILA, MARK A.	GROUP ART UNIT:	1806
SERIAL NO.:	08/064,505	EXAMINER:	PHILLIP GAMBEL
FILED:	MAY 19, 1993		
FOR:	**ANTIBODIES WITH SPECIFICITY FOR MULTIPLE ADHESION MOLECULES**		

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

SIR:

DECLARATION BY JOHN B. STEINBERG

I, JOHN B. STEINBERG declare that:

1. I am currently a cardiothoracic surgeon at Cox South Hospital in Springfield, MO.

2. I received a B.S. degree in Chemistry in 1981, Magna Cum Laude, from the University of Utah, Salt Lake City, Utah. I received an M.D. degree in 1985 from the University of Utah. My clinical training includes 1) general surgery residency (1985-1991), 2) cardiothoracic surgery research fellow (1988-1989), 3) cardiothoracic surgery residency (1991-1994), as detailed in my curriculum vitae (Exhibit 1).

3. I am an Associate Fellow of the American College of Surgeons and am a member of The University of Oklahoma Surgical Society.

4. I am the author of several published articles and abstracts in the area of cardiothoracic surgery and have given many presentations on this topic. (Exhibit 1)

5. I am a practicing clinician specializing in cardiothoracic surgery.

6. I have used an animal model for lung ischemia/reperfusion injury which was developed by Dr. David Kapelanski from the Division of Cardiothoracic Surgery, Department of Surgery at the University of Iowa College of Medicine, Iowa City, Iowa with whom I have collaborated. The induced ischemia in the lung of the animal results in initiation of inflammatory events. Leukocytes migrate and are trapped in areas of inflammation releasing cytotoxic substances causing injury to the organ and possible death to the animal. The extent of injury induced by the ischemia is determined by measurement of inert gas shunt, respiratory gas exchange and survival of the animal.

7. Lung ischemia/reperfusion injury occurs in humans undergoing lung transplantation, and occurs in pulmonary injuries resulting from trauma and sepsis (Adult respiratory distress syndrome). Lung transplantation and pulmonary injuries resulting from trauma and sepsis results in the initiation of inflammatory events. Leukocytes migrate into the organ in the areas of inflammation and release numerous cytotoxic products causing injury to the organ and possible death to the human.

8. It is my opinion that the inflammation-induced injury that occurs in the lung ischemia/reperfusion animal model corresponds with the inflammation-induced injury that occurs in lung transplantation and pulmonary injuries in humans.

9. The lung ischemia/reperfusion animal model has been used to test therapeutics for efficacy in reducing or preventing lung ischemia/reperfusion injury (Exhibit 2 - Kapelanski D.P. et al, Lung Reperfusion Injury is Reduced by Inhibiting a CD18 Dependent Mechanism. Heart Lung Transplant 1993; 12:294-307).

10. The lung ischemia/reperfusion injury sheep model was used to test the efficacy of treatment using the EL-246 antibody. The EL-246 antibody is the identical antibody that is disclosed and claimed in the above-identified patent application. The results of this study are disclosed in the above-identified patent application and are disclose in the article entitled, "Survival in Lung Reperfusion Injury Is Improved By An Antibody That Binds And Inhibits L- and E- Selection", co-authored by myself and H-Z Mae, S.D. Niles, M.A. Jutila and D.P. Kapelanski, The Journal of Heart and Lung Transplantation Vol 13, No. 2, pp 306-318, 1994. (Exhibit 3)

11. The EL-246 antibody demonstrated efficacy in treating sheep to reduce inflammation-induced lung ischemia/reperfusion injury as demonstrated by improved survival, inert gas shunt and respirator gas exchange.

12. One hundred percent of animals treated with the El-246 antibody survived, whereas only 37.5% of the untreated animals and only 33.3% of the DREG 56 treated animals survived.

13. In addition to improved survival, the EL-246 treatment provided other beneficial effects to the treated animals. PaO_2 improved after inflammation-induced lung ischemia/reperfusion injury, less CO_2 retention was noted with animals treated with EL-

246 and shunt (Qs/Qt) was improved after reperfusion injury in animals treated with EL-246.

14. The results also indicate that the EL-246 antibody reached the intended target site, i.e., the lung, and was present in an effective concentration to reduce lung injury.

15. The results show that saturating levels of the EL-246 antibody were maintained for at least six (6) hours after a single intravenous bolus injection. Thus, the EL-246 antibody is not rapidly cleared from the circulation.

16. The sheep that were treated with EL-246 did not suffer any adverse side effects during the six hour experiment.

17. It is my opinion that the efficacy of the EL-246 antibody, as demonstrated by the sheep lung ischemia/reperfusion model is reasonably predictive of efficacy of the EL-246 antibody in humans for treatment of inflammatory-induced injury that occurs in lung transplantation and pulmonary injuries.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made by information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 11/10/94

John B. Steinberg, M.D.

CURRICULUM VITAE

PERSONAL DATA:

NAME: John B. Steinberg **SS:** 520-80-4424

HOME ADDRESS: 2539 South Forrest Heights Avenue
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BUSINESS ADDRESS: Ferrell-Duncan Clinic, Inc.
1001 East Primrose
Springfield, MO 65807

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MARITAL STATUS: Married—Katherine Lynn Steinberg
Children—Lindsey Katherine Steinberg
born December 21, 1990

UNDERGRADUATE EDUCATION:

SCHOOL AND DEGREE: University of Utah; Salt Lake City. UT—B.S. in chemistry, 1981, Magna Cum Laude.

HONORS: Phi Eta Sigma (freshman honor society); Phi Beta Kappa (junior year); Phi Kappa Phi (senior year); Alpha Epsilon Delta (premedical honor society); Lloyd E. Malm Memorial Award (freshman chemistry award); Leon Watters Fellowship (sophomore chemistry award); Leon Watters Fellowship (junior chemistry award); Walter D. and Grace G. Bonner Memorial Award (senior chemistry award).

ACTIVITIES: Freshman year—volunteer work at University of Utah Medical School Hospital; Alpha Epsilon Delta speaker program; chemistry seminars.

MEDICAL EDUCATION:

SCHOOL AND DEGREE: University of Utah School of Medicine; Salt Lake City, UT —M.D., June 1985.

MEMBERSHIPS: American Medical Association.

COMMUNITY INTERESTS AND SERVICE: Summer 1982—Preceptorship in Cheyenne, WY, for six weeks; vacations of MS I and MS II years—worked with an Emergency Room doctor in Cheyenne, WY.

CURRICULUM VITAE

John B. Steinberg

POSTDOCTORAL EDUCATION:

1. **GENERAL SURGERY INTERNSHIP:** University of Oklahoma Health Sciences Center; Oklahoma City, OK—July 1985 through June 1986.
2. **GENERAL SURGERY RESIDENT:** University of Oklahoma Health Sciences Center; Oklahoma City, OK—July 1986 through June 1988.
3. **CARDIOVASCULAR SURGERY RESEARCH FELLOW:** Harvard University; Massachusetts General Hospital; Boston, MA—July 1988 through June 1989.
4. **GENERAL SURGERY RESIDENT:** University of Oklahoma Health Sciences Center; Oklahoma City, OK—July 1989 through June 1990.
5. **GENERAL SURGERY CHIEF RESIDENT:** University of Oklahoma Health Sciences Center; Oklahoma City, OK—July 1990 through June 1991.
6. **CARDIOTHORACIC SURGERY RESEARCH FELLOW:** University of Iowa Hospitals and Clinics; Iowa City, IA—July 1991 through June 1992.
7. **CARDIOTHORACIC SURGERY RESIDENT:** University of Iowa Hospitals and Clinics; Iowa City, IA—July 1992 through June 1994.

HOSPITAL APPOINTMENTS:

1. **ASSOCIATE STAFF SURGEON:** Cox South Hospital, Springfield, MO 65802.

MEMBERSHIPS:

1. Associate Fellow, American College of Surgeons.
2. The University of Oklahoma Surgical Society.

CERTIFICATION:

1. ACLS—1991.
2. ATLS—1991.
3. Diplomate, American Board of Surgery: June 2, 1992 #37356.
4. Board Eligible, American Board of Thoracic Surgery: June 1994.

CURRICULUM VITAE

John B. Steinberg

HONORS/AWARDS:

1. Co-chairman for The 36th Annual Meeting of the Oklahoma Association of House Staff Physicians—May 1990.
2. Chairman for The 37th Annual Meeting of the Oklahoma Association of House Staff Physicians—May 1991.
3. Recipient of the 1991 Aesculapian Award for excellence in teaching clinical sciences from the medical school class of 1992, University of Oklahoma.
4. Recipient of Best Scientific Paper Award at the Residents and Fellows Research Day, University of Iowa Hospitals and Clinics, Iowa City, IA —June 18, 1993.

PUBLICATIONS:

1. Steinberg, J. B., Remis, R.E., Roy, J.B. "Urethral Diverticuli in Women: An Update with a Case Presentation." Military Medicine, 1988; 153: 424—426.
2. Steinberg, J.B., Tuggle, D.W., Postier, R.G. "Adenocarcinoma of the Colon in Adolescents." The American Journal of Surgery 1988; 156: 460—462.
3. Buckner, J.W., Austin, J.C., Steinberg, J.B., Postier, R.G. "Factors Predicting Failure of Medical Therapy for Gastric Ulcers." The American Journal of Surgery 1989; 158: 570—573.
4. Elkins, R.C., Steinberg, J.B., Razook, J.D., Ward, K.E., Overholt, E.D., Thompson, W.M. "Correction of Truncus Arteriosus with Truncal Valvar Stenosis or Insufficiency: Using Two Homografts." Annals of Thoracic Surgery 1990; 50: 728—733.
5. Steinberg, J.B., Doherty, N.E., Munfakh, N.A., Geffin, G.A., Titus, J.S., Hoaglin, D.C., Denenberg, A.G., Daggett, W.M. "Oxygenated Cardioplegia: The Metabolic and Functional Effects of Glucose and Insulin." Annals of Thoracic Surgery 1991; 51: 620—629.
6. Munfakh, N.A., Steinberg, J.B., Titus, J.S., Denenberg, A.G., O'Keefe, D.D., Daggett, W.M., Geffin, G.A. "Protection of the Hypertrophied Myocardium by Crystalloid Cardioplegia." The Journal of Surgical Research 1991; 51: 447—456.
7. Steinberg, J.B., Nickell, S.A., Jacocks, M.A., Stelzer, P. "Replacement of the Abdominal Aorta with an Aortic Homograft in a Patient with an Aortic Dissection." Annals of Vascular Surgery 1991; 5: 538—541.

CURRICULUM VITAE
John B. Steinberg

PUBLICATIONS, Cont.:

8. Steinberg, J.B., Reynolds, T.R., Postier, R.G. "Adenosquamous Cell Carcinoma of the Colon." Surgical Rounds 1992; 15: 297—298.
9. Byers, J.M., Steinberg, J.B., Postier, R.G. "Repair of Parastomal Hernias Using Polypropylene Mesh." Archives of Surgery 1992; 127: 1246—1247.
10. Allyn, J.W., Teplick, R., Steinberg, J.B., Munfakh, N.A., Geffin, G.A., Daggett, W.M. "Norepinephrine Increases the Economy of Pressure Development in Isolated Canine Hearts." American Journal of Physiology 1992; 263: H715—H721.
11. Niles, S.D., Ploessl, J., Sutton, R.G., Steinberg, J.B. "Oxygenator Failure Due to Contact with Bathing Alcohol: A Case Report." Journal of Extra-Corporeal Technology 1992; 24: 69—71.
12. Steinberg, J.B., Kresowik, T.F., Behrendt, D.M. "Prophylactic Myocardiorevascularization Based on Dipyridamole–Thallium Scanning Before Peripheral Vascular Surgery." Journal of Cardiovascular Surgery. 1993; 1: 552—557.
13. Steinberg, J.B., Kapelanski, D.P., Olson, J.D., Weiler, J.M. "Cytokine and Complement Levels in Patients Undergoing Cardiopulmonary Bypass." The Journal of Thoracic and Cardiovascular Surgery 1993; 106: 1008—1016..
14. Steinberg, J.B., Johnson, E.R., Benda, J.A., Lanza, L.A. "Primary Leiomyosarcoma of the Thoracic Aorta Presenting as a Contained Rupture." Annals of Thoracic Surgery 1993; 56: 1387—1389.
15. Steinberg, J.B., Jacocks, M.A. "May–Thurner Syndrome: A Previously Unreported Variant and Review of the Literature." Annals of Vascular Surgery 1993; 7: 577—581.
16. Steinberg, J.B., Mao, H.Z., Niles, S.D., Jutila, M.A., Kapelanski, D.P. "Survival in Lung Reperfusion Injury Is Improved by an Antibody That Binds and Inhibits L- and E-Selectin." The Journal of Heart and Lung Transplantation 1994: 13: 306—318:
17. Delius, R.E., Steinberg, J.B., L'Ecuyer, T.J., Doty, D.B., Behrendt, D.M., "Long-term Follow-up of Extended Aortoplasty for Supravalvular Aortic Stenosis." In press, The Journal of Thoracic and Cardiovascular Surgery.
18. Molin, L.J., Steinberg, J.B., Lanza, L.A., "Video Assisted Thoracic Surgery (VATS) Increases Procedural Costs in Patients Undergoing Elective Lung Biopsy for Interstitial Lung Disease." In press, Annals of Thoracic Surgery

CURRICULUM VITAE
John B. Steinberg

ABSTRACTS:

1. Steinberg, J.B., Tuggle, D.W., Postier, R.G. "Adenocarcinoma of the Colon in Adolescents." The booklet of The Southwestern Surgical Congress, 40th Annual Meeting, April 10–13, 1988: 29.

2. Buckner, J.W., Austin, J.C., Steinberg, J.B., Postier, R.G. "Factors Predicting Failure of Medical Therapy for Gastric Ulcers." The booklet of The Southwestern Surgical Congress, 41st Annual Meeting, April 23–26, 1989: 49.

3. Allyn, J.W., Teplick, R., Steinberg, J.B., Munfakh, N.A., Geffin, G.A., Daggett, W.M. "Norepinephrine Does Not Result in Oxygen–Wasting in Isolated Canine Hearts." The booklet of The Society of Cardiovascular Anesthesiologists, 12th Annual Meeting, May 13 –16, 1990.

4. Steinberg, J.B., Doherty, N.E., Munfakh, N.A., Geffin, G.A., Titus, J.S., Hoaglin, D.D., Denenberg, A.G., Daggett, W.M. "The Addition of Glucose and Insulin to an Oxygenated Cardioplegic Solution." Journal of Molecular and Cellular Cardiology 1990; 22: 13.

5. Munfakh, N.A., Steinberg, J.B., Geffin, G.A., Titus, J.S., O'Keefe, D.D., Daggett, W.M. "Protection of the Hypertrophied Myocardium by Crystalloid Cardioplegia." The booklet of The Association for Academic Surgery, 24th Annual Meeting, November 14–17, 1990: 24.

6. Byers, J.M., Steinberg, J.B., Postier, R.G. "Repair of Parastomal Hernia Using Marlex Mesh." The booklet of The Southwestern Surgical Congress, 43rd Annual Meeting, April 21–24, 1991: 5.

7. Steinberg, J.B., Kapelanski, D.P., Jutila, M.A., Niles, S.D., Mao, H.Z. "Survival in Lung Reperfusion Injury Is Improved by Inhibiting L-Selectin Mediated Adhesion.." The Journal of Heart and Lung Transplantation 1993; 12: S66.

8. Molin, L.J., Steinberg, J.B., Lanza, L.A. "VATS Increases Procedural Costs in Patients Undergoing Lung Biopsy for Pulmonary Fibrosis." The booklet of The Society of Thoracic Surgeons, 30th Annual Meeting, January 31–February 2, 1994: 63.

9. Delius, R.E., Steinberg, J.B., L'Ecuyer, T.J., Doty, D.B., Behrendt, D.M., "Long-term Follow-up of Extended Aortoplasty for Supravalvular Aortic Stenosis." The booklet of The American Association for Thoracic Surgery, 74th Annual Meeting, April 24—27, 1994: 114.

CURRICULUM VITAE

John B. Steinberg

PRESENTATIONS:

1. Steinberg, J.B., Tuggle, D.W., Postier, R.G. "Adenocarcinoma of the Colon in Adolescents." Presented at the 40th annual meeting of The Southwestern Surgical Congress. Phoenix, AZ, April 10, 1988.
2. Steinberg, J.B., Nickell, S.A., Jacocks, M.A., Stelzer, P. "Replacement of the Abdominal Aorta with an Aortic Homograft: A Case Report." Presented at the 42nd annual meeting of The Southwestern Surgical Congress. La Quinta, CA, April 24, 1990.
3. Steinberg, J.B., Doherty, N.E., Munfakh, N.A., Geffin, G.A., Titus, J.S., Hoaglin, D.C., Denenberg, A.G., Daggett, W.M. "The Addition of Glucose and Insulin to Oxygenated Cardioplegic Solution." Poster presentation at the Journal of Molecular and Cellular Cardiology International Society for Heart Research. Myocardial Preservation into the 21st Century. The Oxford International Symposium. Oxford, England, August 12–15, 1990.
4. Byers, J.M., Steinberg, J.B., Postier, R.G. "Repair of Parastomal Hernia Using Marlex Mesh." Poster presentation at the 43rd annual meeting of The Southwestern Surgical Congress. Las Vegas, NV, April 21–24, 1991.
5. Steinberg, J.B. "Repair of Parastomal Hernias." Oklahoma Surgical Association Scientific Program. Sheraton Century Center, Oklahoma City, OK, May 10, 1991.
6. Steinberg, J.B. "Pancreatic Cancer." Surgical Grand Rounds, University of Oklahoma Health Sciences Center. Oklahoma City, OK, June 15, 1991.
7. Steinberg, J.B. "Chest Trauma." Advanced Trauma Management Course. University of Iowa Hospitals and Clinics. Iowa City, IA, October 3–4, 1991.
8. Steinberg, J.B. "The Pathophysiologic Effects of Cardiopulmonary Bypass." Cardiothoracic Surgery Grand Rounds, University of Iowa Hospitals and Clinics. Iowa City, IA, November 17, 1991.
9. Steinberg, J.B. "IV Techniques." ACLS Course. The University of Iowa Hospitals and Clinics. Iowa City, IA, February 17, 1992.
10. Steinberg, J.B. "Screening for Cardiac Disease Prior to Peripheral Vascular Surgery: The Use of the Dipyridamole–Thallium Scan." Cardiothoracic Surgery Grand Rounds, University of Iowa Hospitals and Clinics. Iowa City, IA, March 14, 1992.
11. Steinberg, J.B., Jacocks, M.A. "May–Thurner Syndrome: Surgical Correction of an Unusual Variant." Presented at the 44th annual meeting of The Southwestern Surgical Congress. Scottsdale, AZ, April 28, 1992.

CURRICULUM VITAE

John B. Steinberg

PRESENTATIONS, Cont.:

12. Parsons, B.D., Mantor, C., Steinberg, J.B., Cannon, J.P., Postier, R.G. "Pylorus Preservation: Is It Appropriate in Resection for Pancreatic Cancer?" Poster presentation at the 44th annual meeting of The Southwestern Surgical Congress. Scottsdale, AZ., April 26 –29, 1992.

13. Steinberg, J.B. "Intraaortic Balloon Counterpulsation." Cardiothoracic Surgery Grand Rounds, University of Iowa Hospitals and Clinics. Iowa City, IA, October 10, 1992.

14. Steinberg, J.B. "Survival in Lung Reperfusion Injury Is Improved by Inhibiting L-Selectin Mediated Adhesion." Presented at the 13th annual meeting of The International Society for Heart and Lung Transplantation. Boca Raton, FL, March 31, 1993.

15. Steinberg, J.B. "Survival in Lung Reperfusion Injury Is Improved by Inhibiting L-Selectin Mediated Adhesion." Presented at Residents and Fellows Research Day. University of Iowa Hospitals and Clinics. Iowa City, IA, June 18, 1993.

16. Steinberg, J.B., Fieselmann, J.F., Lanza, L.A. "Orthodeoxia and Platypnea Following Right Pneumonectomy." Presented at the 30th annual meeting of The Society of Thoracic Surgeons. New Orleans, LA, February 2, 1994.

17. Molin, L.J., Steinberg, J.B., Lanza, L.A. "VATS Increases Procedural Costs in Patients Undergoing Lung Biopsy for Pulmonary Fibrosis." Poster presentation at the 30th annual meeting of The Society of Thoracic Surgeons. New Orleans, LA, January 31–February 2, 1994.

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Lung Reperfusion Injury Is Reduced by Inhibiting a CD18-dependent Mechanism

David P. Kapelanski, MD, Atsushi Iguchi, MD, Scott D. Niles, BS, and Hui-Zhen Mao, MD

CD18 designates a component of a leukocyte surface glycoprotein complex that mediates endothelial adherence. To determine whether interference with CD18-dependent leukocyte adhesion modifies reperfusion injury, we transplanted 16 canine left lungs after 4-hour preservation with modified Euro-Collins solution. Anti-canine CD18 monoclonal antibody (R15.7, 1 mg/kg, intravenously) was administered to eight lung recipients 5 minutes before reperfusion; eight control recipients were not treated. Ventilation was identical in donor-recipient pairs (tidal volume, 600 ml; fraction of inspired oxygen, 0.53; positive end-expiratory pressure, 5 cm H_2O). Respiratory and inert gas exchange and hemodynamics were assessed in left lung donors one-half hour after right lung exclusion and in allograft recipients at 0.5, 1.5, 2.5, 3.5, and 6.0 hours after transplantation and right lung exclusion. Reperfusion injury was evident in both recipient groups at 6 hours after transplantation, but inert gas shunt was lower in monoclonal antibody–treated dogs (13% ± 6%) than in controls (30% ± 17%, $p < 0.05$), comparisons of arterial blood gases in monoclonal antibody recipients (PaO_2, 209 ± 83 mm Hg; $PaCO_2$, 45 ± 7 mm Hg) and controls (PaO_2, 108 ± 54, $p < 0.05$; $PaCO_2$, 64 + 25, $p < 0.05$) at 6 hours indicated that monoclonal antibody administration distinctly improved respiratory gas transfer. Gravimetric lung water was less in monoclonal antibody recipients (5.78 ± 1.01 ml/kg) than in controls (8.02 ± 1.90 ml/kg, $p < 0.05$), but lung compliance at 6 hours was equally reduced in monoclonal antibody recipients (40 ± 9 ml/cm H_2O) and in controls (39 ± 7 ml/cm H_2O, p = not significant). Pulmonary vascular resistance doubled immediately after transplantation but was identical in monoclonal antibody–treated dogs (890 ± 168 dynes · sec · cm^{-5}) and in controls (874 ± 162 dynes · sec · cm^{-5}, p = not significant) at 6 hours. We conclude that inhibition of CD18-dependent leukocyte function attenuates the development of both shunt and abnormal respiratory gas exchange in lung reperfusion injury. Significant physiologic abnormalities occurred despite R15.7 treatment and may represent inadequate preservation or the effect of CD18-independent adhesion mechanisms. J HEART LUNG TRANSPLANT 1993;12:294-307.

The β_2 integrins are a group of cell surface glycoproteins that are functionally characterized by their capacity to mediate adhesion between leukocytes and other leukocytes; between leukocytes and other cells of non-myeloid lineage, including endothelium; and between leukocytes and nonsolubilized components of the extracellular matrix, including inactivated C3b, fibrinogen, and factor X.[1-4] Structurally, the β_2 integrins are distinguished by a common β-subunit (CD18) noncovalently associated with one of three homologous α-subunits (CD11a, CD11b, and CD11c), thus forming three distinct heterodimers: lymphocyte function–associated antigen-1 (CD11a/CD18); macrophage-1 (CD11b/CD18); and p150/95 (CD11c/CD18). Although CD11a/CD18 is found on essentially all myeloid derivatives, surface expression of CD11b/CD18 and CD11c/CD18 by nonmalignant tissue is generally confined to phagocytic cells and certain lymphocyte populations. The β_2 integrins are

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Presented at the Twelfth Annual Meeting and Scientific Sessions of the International Society for Heart and Lung Transplantation, San Diego, California, April 2-4, 1992.

Submitted May 4, 1992; accepted September 1, 1992.

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therefore normally restricted to cells capable of participating in an immune response.

The cell surface counterreceptors for the β_2 integrins are monomeric glycoproteins.[5] Two such intracellular adhesion molecules (ICAM-1 [CD54] and ICAM-2) have been described, and presumptive evidence has been found for the existence of a third ICAM.[4,5] As is the case with most other members of the immunoglobulin superfamily, the ICAMs are characterized by the presence of repetitive immunoglobulin-like domains, there being five such sequences in ICAM-1 and two in ICAM-2. In unstimulated adult and fetal human tissue, ICAM-2 is expressed only by interstitial lymphoid cells and by endothelium, including pulmonary capillary endothelium.[4] ICAM-1 is much more broadly distributed and in particular has been identified on pulmonary capillary endothelium, alveolar lining cells, type I and type II pneumocytes, and alveolar macrophages. Although ICAM-1 binds to both CD11a/CD18 and CD11b/CD18, ICAM-2 binds exclusively to CD11a/CD18.[4] In addition a quantitative difference exists in the binding affinity of CD11a/CD18 for the ICAMs that preferentially favor an interaction with ICAM-1.[4]

β_2 integrin–mediated adhesion is a highly controlled process. When neutrophils are activated in vitro by C5a, leukotriene B_4, platelet-activating factor, or tumor necrosis factor, CD11b/CD18 expression is up-regulated within minutes by translocation of sequestered dimer from intracellular storage granules to the surface membrane.[6-9] After a variable interval that is partly dependent on the specific inflammatory stimulus, surface expression reverts to basal levels. Conversely, activation of the T-cell receptor does not alter lymphocyte CD11a/CD18 expression but rather induces a rapid and transient increase in CD11a/CD18 avidity for ICAM-1, which can be reinvoked by repetitive stimulation; and evidence exists that neutrophil CD11b/CD18 affinity for ICAM-1 is comparably regulated.[5,6,10,11] Similarly, ICAM-1 expression by unstimulated endothelial cells is low but can be induced by several substances (tumor necrosis factor, interleukin-1 [IL-1], lipopolysaccharide, or interferon-γ).[4,12-14] Enhanced expression requires *de novo* RNA and protein synthesis, is initially detectable within 4 hours of stimulation, and peaks at about 24 hours. Unlike ICAM-1, however, ICAM-2 is noninducible, though surface expression by unstimulated endothelial cells is approximately tenfold higher than ICAM-1 expression. Current evidence indicates that avidity of the ICAMs is not regulated.

Experimental data is accumulating that indicates interference with β_2 integrin–mediated leukocyte adhesion can limit the development of postischemic injury. Administration of an anti-CD18 monoclonal antibody (MAb) (60.3) immediately before reperfusion diminished the extent of tissue necrosis in rabbit ears subjected to 10 hours of ischemia.[15] An associated reduction occurred in neutrophil influx, microvascular thrombosis, interstitial edema, and hemorrhage in the ears of treated animals, but this effect was not further enhanced when MAb was administered before ischemia and repeated before reperfusion. Similarly, administration of an anti-CD18 MAb (60.3) (5 minutes before reperfusion and 12 hours after onset of reperfusion) had no immediate (10 minutes) effect on the maldistribution of pulmonary flow in rabbit lungs reperfused after 24-hour *in situ* pulmonary artery occlusion, although the perfusion pattern normalized in treated but not control animals by 24 hours after the onset of reperfusion.[16] In this same model, MAb recipients had fewer leukocytes and neutrophils in lavage fluid sampled from either the ischemic or the contralateral lung at 24 hours of reperfusion. In a comparable model of 24-hour *in situ* rabbit lung ischemia, ICAM-1 expression in the ischemic lung increased fourfold within the initial 30 minutes of reperfusion.[17] Treatment with either anti-ICAM-1 MAb (RR1/1) or anti-CD18 MAb (1B4) 1 hour before reperfusion prevented an increase in pulmonary capillary filtration coefficient. Both MAbs reduced lung water accumulation by an equivalent amount, although neither MAb modified the characteristic increase in pulmonary artery pressure that develops during reperfusion. These investigators documented an increase in tissue myeloperoxidase activity in the ischemic and in the contralateral lungs of control rabbits and further showed that both of the MAbs individually abrogated this increase in the contralateral lung, but only reduced myeloperoxidase activity in the ischemic lung.

From the foregoing, we reasoned that we could specifically interfere with β_2 integrin–mediated leukocyte adhesion and thereby modify the pattern of acute physiologic abnormalities that characterize lungs subjected to hypothermic preservation and transplantation. We proposed to test this hypothesis in experimental lung transplant recipients by administering an anti-CD18 antibody immediately before the onset of reperfusion. We determined to assess the effect of this treatment by serial evaluation of hemodynamics, lung compliance, and respiratory and inert gas exchange in donors and recipients and

by postmortem measurement of lung water. Furthermore, if an effect of antibody was shown, we intended to use these data to identify the physiologic parameters that influence abnormal respiratory gas exchange in this injury.

METHODS

General methods

General anesthesia was induced in unconditioned mongrel dogs (weight, 21 to 30 kg) with thiopental sodium (500 mg, intravenously). A deep plane of surgical anesthesia was maintained in donors and recipients by continuous administration of fentanyl citrate (0.5 mg/hr, intravenously). Complete paralysis was sustained in donors and recipients by continuous administration of pancuronium bromide (5.0 mg/hr, intravenously).

Volume-controlled ventilation (tidal volume, 600 ml; fraction of inspired oxygen, 0.53; inspiratory:expiratory ratio, 1:1; positive end-expiratory pressure, 5.0 cm H_2O) (608 ventilator; Harvard Apparatus, Inc., S. Natick, Mass.; air-oxygen mixer; Sechrist Industries, Inc., Anaheim, Calif.; positive end-expiratory pressure valve; Boehringer Laboratories, Inc., Norristown, Pa.) was delivered through an 8-mm cuffed endotracheal tube. The ventilator rate (10 to 15/min) in donors was adjusted before right lung exclusion to achieve an arterial carbon dioxide tension ($PaCO_2$) of approximately 30 mm Hg. These ventilator settings were maintained for the remainder of the experiment.

The surface electrocardiogram was continuously displayed using standard limb leads (ES2000; Gould Inc., Test & Msmt. Rec. Syst. Div., Valley View, Ohio). Intravascular pressures were continuously monitored using fluid-filled catheters and mercury-calibrated strain gauges (P23XL; Gould Inc.). The difference between airway and atmospheric pressure in the open chest dog was continuously monitored at the endotracheal tube inlet with a water calibrated differential pressure transducer (DP45-16-2114; Validyne Engineering Corp., Northridge, Calif.) Lung compliance was estimated from the quotient: (peak inspiratory pressure − positive end-expiratory pressure)/tidal volume. Cardiac output was determined by thermodilution (American Edwards Laboratories, Deerfield, Ill.), and the mean of triplicate measurements was indexed to body weight. Expired minute ventilation was assessed by timed collection with a calibrated respirometer (Wright Manufacturing Co., Arlington, Tenn.) and indexed to body weight. Hematocrit was measured in arterial blood with an impedance electrode (NOVA Biomedical Corporation, Waltham, Mass.). Total hemoglobin, hemoglobin saturation, and blood oxygen content were determined by optical absorbance (Radiometer America, Inc., West Lake, Ohio). Oxygen and carbon dioxide tension in blood and gas were measured with calibrated micro-Clark and Severinghaus electrodes (NOVA Biomedical Corporation) at 37° C. Blood pH was measured using a calibrated Sanz electrode (NOVA Biomedical Corporation) at 37° C. Blood gas tensions and pH were corrected to body temperature, pressure, saturated, using the algorithms of Thomas.[18] Oxygen consumption ($\dot{V}O_2$) was calculated from the arterial and mixed venous oxygen content difference and cardiac output. Carbon dioxide elimination ($\dot{V}CO_2$) was calculated from carbon dioxide tension in mixed-expired gas and expired minute ventilation, assuming carbon dioxide was not present in inspired gas. $\dot{V}O_2$ and $\dot{V}CO_2$ were indexed to body weight and converted to STPD. Gravimetric lung water was determined by comparison of lung weight before and after desiccation at 40° C.

Continuous ventilation:perfusion ($\dot{V}A/\dot{Q}$) distributions were estimated using the multiple inert gas elimination technique.[19,20] In brief, a preparation containing six volatile agents (SF_6, ethane, cyclopropane, halothane, diethyl ether, and acetone) dissolved in 0.9% saline solution was continuously infused (4 ml/min) into the left femoral vein. During a physiologic steady state defined by stable blood pressure and heart rate, matching 10-ml arterial and mixed venous blood samples were simultaneously aspirated over 1 minute. A matching expired gas specimen was collected from a thermostatically controlled mixing chamber after a delay appropriate to the volume of the mixing chamber and expired minute ventilation. Blood and expired gas specimens for inert gas analysis were collected in duplicate. The inert gases were extracted from blood by equilibration with nitrogen at 37° C. Inert gas concentrations in the gas phase were determined by gas chromatography, using megabore columns (DB1, J & W Scientific, Folsom, Calif.; Pora Plot U, Chrompack, Middleburg, The Netherlands) and a flame ionization and electron capture detector–equipped machine (Hewlett-Packard Co., Medical Products Group, Andover, Mass.) The individual inert gas data from the duplicate blood and expired gas samples were averaged, then used as input to the enforced smoothing algorithm of Evans and Wagner ($z = 40$).[21]

Experimental Protocol

Left lung allografts were procured from supine donors, anesthetized, and monitored as previously

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TABLE I Arterial oxygen and carbon dioxide tension

		Recipient							
	Donor	0.5 Hours	1.5 Hours	2.5 Hours	3.5 Hours	6.0 Hours	p* (η^2)	Group mean	p† (η^2)
Pao_2 (mm Hg)									
Control	311 ± 25	208 ± 96	169 ± 81	128 ± 74	112 ± 65	108 ± 54	0.131	173 ± 98	0.019
MAb	317 ± 14	236 ± 95	246 ± 62	220 ± 78	196 ± 86	209 ± 83		237 ± 81	0.335
$Paco_2$ (mm Hg)									
Control	28 ± 3	43 ± 17	49 ± 19	54 ± 17	55 ± 17	64 ± 25	0.032	49 ± 20	0.084
MAb	31 ± 5	36 ± 7	36 ± 6	40 ± 8	42 ± 6	45 ± 7	0.240	38 ± 8	

MAb, Monoclonal anti-canine CD18 antibody R15.7; Pao_2, arterial oxygen tension; η^2, treatment magnitude (tabulated below *p* value for significant group effects); $Paco_2$, arterial carbon dioxide pressure.
Tabulated data indicate the mean and standard deviation.
Donors were assessed 0.5 hours after right lung exclusion; recipients were assessed after reperfusion and right lung exclusion at the times specified.
**p* for within subjects group effect using Greenhouse-Geisser procedure.
†*p* for between subjects group effect.

described. After median sternotomy and systemic heparinization (300 units/kg, intravenously), the right main pulmonary artery was ligated, and the right main bronchus was clamped. Physiologic data were recorded after a 30-minute equilibration period. The superior and inferior vena cavae were then ligated, and the donor was exsanguinated. The left lung was simultaneously flush-perfused with 2 liters of cold (4° C) Euro-Collins solution, supplemented with $MgSO_4$ (8 mEq/L) and prostaglandin E_1 (500 units/L) (Upjohn Company, Kalamazoo, Mich.). The lungs were inflated with room air to a pressure of 25 cm H_2O; the trachea was clamped, and the heart and lungs were excised en bloc. The inflated graft was stored in cold 0.9% saline solution for 3 hours.

Lung recipients were anesthetized and monitored as described previously. After thoracotomy and left pneumonectomy, the allograft was removed from hypothermic storage, prepared, and implanted in standard fashion. Five minutes before left lung reperfusion, a bolus infusion (1.0 mg/kg, intravenously, in 25 ml 0.9% saline solution) of R15.7, an immunoglobulin-G1 anti-canine CD18 antibody, was administered to eight lung recipients.[22] Eight control allograft recipients were not treated. The left bronchus was unclamped, and both lungs were manually recruited with several large volume breaths. Intravascular air was displaced, and the graft was reperfused after 4 hours of ischemia. The recipient was then positioned supine. After a 10-minute interval of dual lung perfusion and ventilation, the right main bronchus was clamped at end-expiration, and the recipient right pulmonary artery was ligated. Physiologic data were recorded at 0.5, 1.5, 2.5, 3.5, and 6.0 hours after the onset of reperfusion (4.5, 5.5, 6.5, 7.5, and 10.0 hours after donor determinations). Sodium bicarbonate was administered as necessary to maintain arterial pH (pHa) above 7.30. Recipient airway secretions were aspirated as necessary but always preceded physiologic measurements by a minimum of 20 minutes. Heating blankets and warming lamps were used to minimize variation in recipient core temperature. Purified R15.7 was provided by Robert Rothlein, PhD, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Conn.

Euthanasia was performed at the end of each experiment by a potassium chloride overdose in completely anesthetized dogs. Death was verified before autopsy, at which time the left lung was excised, and lung water was determined. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Data Analysis

All tabulated data are presented as the mean ± standard deviation. Gravimetric lung water was compared by an independent sample *t*-test, assuming unequal group variances.[23] Serially sampled data were compared with a two-factor (group × time) repeated measures analysis of variance, using the Greenhouse-Geisser procedure to protect against violation of the assumption of homogeneity of covariance.[24] In all instances, the critical alpha for main effects was 0.05. Whenever the main analysis

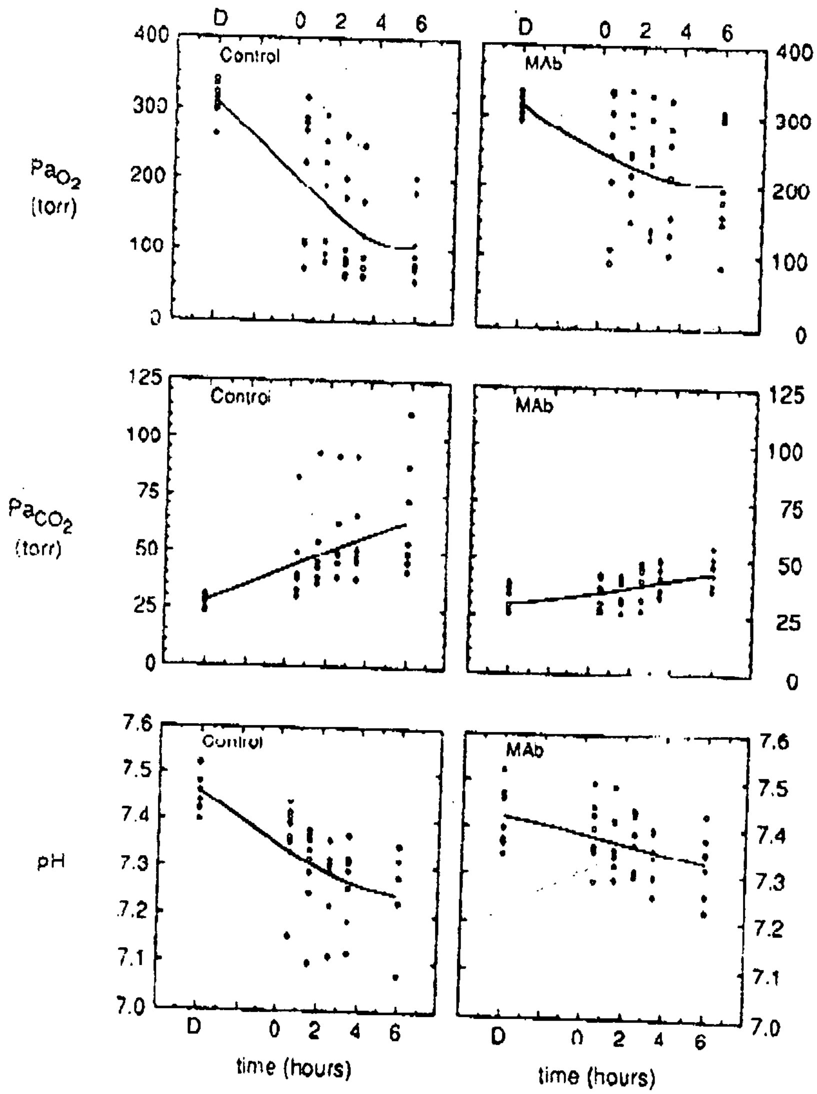

FIGURE 1 Arterial oxygen and carbon dioxide tension (PaO_2, $PaCO_2$) and pH in control (left) and R15.7 (MAb)-treated recipients (right) are displayed on the ordinates. Time is displayed on the abscissae, from the initial determination in donors through the 6-hour period of reperfusion. Smoothing by distance weighted least squares with a tension factor of 0.125. Administration of MAb maintained PaO_2 at a higher level than in control recipients and reduced the rate at which hypercapnia and respiratory acidosis developed after hypothermic preservation and transplantation.

indicated an effect of group within subjects, that is, non-parallel control and MAb profiles, simple contrasts were performed at each time in an attempt to identify specific times at which MAb recipients differed from controls, using an adjusted alpha of $0.05 \div 6$ times $= 0.008$ to preserve the overall experimental alpha of 0.05.[24,25] Treatment magnitude (η^2), which estimates the proportion of variance in a dependent variable (for example arterial oxygen tension) attributable to an independent variable (that is, group), was calculated from the quotient[26]: SS_{effect} ($SS_{effect} + SS_{error}$), where SS designates sum of squares. Finally, to investigate the influence of changing physiologic parameters on respiratory gas exchange in this model, the dependence of either arterial oxygen or carbon dioxide tension (PaO_2, $PaCO_2$) and other serially determined variables was assessed using the Spearman rank correlation (r_s), a distribution free measurement of association.[27] In these evaluations, a conservative critical alpha of 0.001 was used, that is, $|r_s| > 0.332$ (two-tailed test).

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TABLE II Inert gas shunt and deadspace

	Donor	Recipient							
		0.5 Hours	1.5 Hours	2.5 Hours	3.5 Hours	6.0 Hours	p* (η^2)	Group mean	p† (η^2)
$\dot{Q}S/\dot{Q}T$ (%)									
Control	4 ± 3	10 ± 7	19 ± 11	31 ± 18	32 ± 17	30 ± 17	0.001	21 ± 17	0.015
MAb	3 ± 2	8 ± 5	11 ± 4	13 ± 6	14 ± 6	13 ± 6	0.264	10 ± 6	0.274
$\dot{V}D/\dot{V}E$ (%)									
Control	60 ± 5	63 ± 6	62 ± 6	63 ± 5	64 ± 6	66 ± 5	0.125	63 ± 6	0.421
MAb	59 ± 5	68 ± 6	66 ± 6	66 ± 7	65 ± 6	67 ± 4		65 ± 6	

$\dot{Q}S/\dot{Q}T$, Inert gas shunt; $\dot{V}D/\dot{V}E$, inert gas deadspace; other abbreviations defined in Table 1.
Tabulated data indicate the mean and standard deviation.
Donors were assessed 0.5 hours after right lung exclusion; recipients were assessed after reperfusion and right lung exclusion at the times specified.
*p for within subjects group effect using Greenhouse-Geisser procedure.
†p for between subjects group effect.

RESULTS

PaO_2 was comparable in both groups of donors (control, 311 ± 25 mm Hg; MAb, 317 ± 14 mm Hg) (Table 1, Figure 1). PaO_2 in control recipients declined progressively throughout reperfusion, although the largest reduction occurred within the initial 30 minutes (208 ± 96 mm Hg). PaO_2 in MAb-treated recipients was similarly reduced at 30 minutes of reperfusion (236 ± 95 mm Hg) but changed minimally thereafter. Although no difference was noted when the group PaO_2 profiles were compared, the group mean PaO_2 was higher in MAb (237 ± 81 mm Hg) than in control (173 ± 98 mm Hg) recipients. Administration of MAb thus attenuated an aggregate reduction in PaO_2 characteristic of unmodified injury but had negligible effect on that component of the PaO_2 reduction that occurred during the initial minutes of reperfusion.

With relatively high minute ventilation, lung donors were hypocapnic and mildly alkalotic (Table I, Figure 1). Hypercapnia and respiratory acidosis developed in all recipients as a linear function of time, although the slopes of both trends were reduced in MAb-treated subjects. When either $PaCO_2$ or pHa were contrasted between groups at specific times, however, none of these comparisons exceeded the adjusted critical alpha of 0.008. Similarly, no effect of MAb was identified when either the group mean $PaCO_2$ or pHa was compared. Administration of MAb therefore altered a trend of progressive respiratory acidosis after transplantation and, within the bounds of the experimental and analytic methods, asserted this effect in a consistent manner throughout the period of observation.

Inert gas shunt ($\dot{Q}S/\dot{Q}T$) was normal in donor lungs (control, 4% ± 3%; MAb, 3% ± 2%) (Table II, Figure 2). $\dot{Q}S/\dot{Q}T$ doubled (10% ± 7%) in control lungs during the initial 30 minutes after transplantation and reached a peak (32% ± 17%) at 3.5 hours after reperfusion. The evolution of $\dot{Q}S/\dot{Q}T$ in MAb lungs followed a similar pattern, albeit at a slower rate, increasing twofold over the first 30 minutes of reperfusion (8% ± 5%) and attaining a plateau (14% ± 6%) by 3.5 hours. The control and MAb profiles exhibited different linear trends, but, even though the group mean $\dot{Q}S/\dot{Q}T$ was substantially higher in controls (21% ± 17%) than in MAb lungs (10% ± 6%), simple contrasts failed to identify any single time at which $\dot{Q}S/\dot{Q}T$ differed between MAb and control lungs. Thus although administration of MAb did not preclude $\dot{Q}S/\dot{Q}T$ formation, it reduced the rate at which $\dot{Q}S/\dot{Q}T$ developed and constrained the maximum level of $\dot{Q}S/\dot{Q}T$.

The mean $\dot{V}A/\dot{Q}$ of the perfusion ($\dot{Q}$) distribution (mean $\dot{Q}$) and the log standard deviation of the $\dot{Q}$ distribution (std dev $\dot{Q}$) are two indexes of $\dot{V}A/\dot{Q}$ heterogeneity that exclude the influence of $\dot{Q}S/\dot{Q}T$. Both of these indexes were normal in donor lungs (Figure 2). A two-thirds reduction in mean $\dot{Q}$ was identified in both groups within the first 30 minutes of reperfusion, and at that same time a small increase in std dev $\dot{Q}$ was observed. Although the reduction of mean $\dot{Q}$ was sustained, the std dev $\dot{Q}$ gradually reverted to the level measured in donors. MAb administration had no effect on the net reduction in mean $\dot{Q}$, nor did it influence the overall trend in the log std dev $\dot{Q}$. Although by either of these parameters $\dot{V}A/\dot{Q}$ mismatch increased during reperfusion, the net effect on oxygen exchange appeared comparatively small, because the correlation between PaO_2 and mean $\dot{Q}$ (r_s = 0.357) was

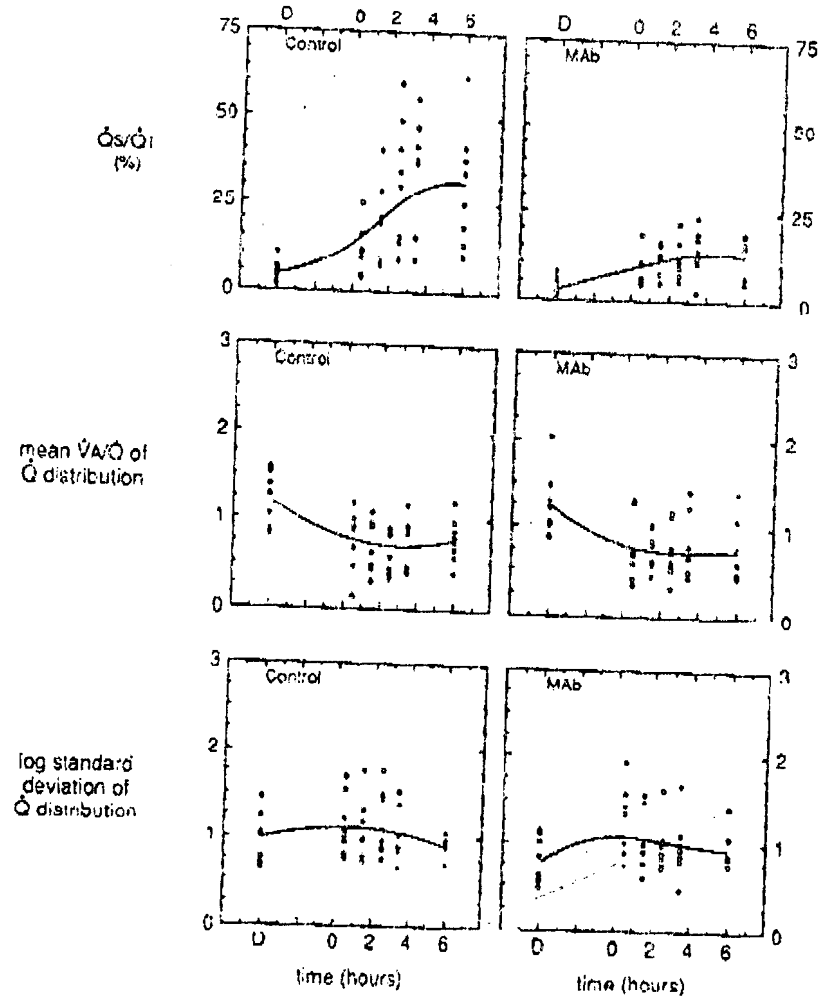

FIGURE 2 Inert gas shunt ($\dot{Q}s/\dot{Q}_T$), the mean ventilation:perfusion ratio ($\dot{V}A/\dot{Q}$) of the perfusion ($\dot{Q}$) distribution, and the log standard deviation of the perfusion distribution in controls (**left**) and R15.7 (MAb)-treated recipients (**right**) are displayed on the ordinates. Time is displayed on the abscissae. Smoothing as described in Figure 1. Administration of MAb limited the deterioration in respiratory gas exchange principally by reducing the formation of $\dot{Q}s/\dot{Q}T$ but had no effect on other characteristics of the perfusion distribution.

substantially lower than that between PaO_2 and $\dot{Q}s/\dot{Q}T$ ($r_s = -0.911$) (Table III). Conversely, the association between $PaCO_2$ and mean $\dot{Q}$ ($r_s = -0.619$) was equivalent to that between $PaCO_2$ and $\dot{Q}s/\dot{Q}T$ ($r_s = 0.695$), although the log std dev $\dot{Q}$ was unrelated to either PaO_2 or $PaCO_2$.

With the relatively large tidal volumes used during single lung ventilation, inert gas deadspace ($\dot{V}D/\dot{V}E$) was abnormally elevated in donors (control, 60% ± 5%; MAb, 59% ± 5%) (Table II). $\dot{V}D/\dot{V}E$ increased in both groups within 30 minutes of transplantation (control, 63% ± 6%; MAb, 68% ± 6%), and these changes persisted for the remainder of the experiment. Although the absolute increase in $\dot{V}D/\dot{V}E$ was confirmed by the main analysis, no treatment effect was noted when $\dot{V}D/\dot{V}E$ was compared in MAb and control subjects. Because $\dot{V}D/\dot{V}E$ was not associated with either PaO_2 or $PaCO_2$, the effect of this increase in $\dot{V}D/\dot{V}E$ on respiratory gas exchange appears to have been inconsequential.

Despite controlled ventilation and continuous provision of anesthetic and paralytic agents, both $\dot{V}O_2$ and $\dot{V}CO_2$ increased during reperfusion (Figure 3). Even though MAb reduced the slope of the $\dot{V}O_2$

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TABLE III Determinants of arterial oxygen and carbon dioxide tension

	Spearman rank correlation	
	Pao_2	$Paco_2$
Expired minute ventilation, indexed to body weight	0.086	−0.204
Cardiac output, indexed to body weight	−0.222	0.289
Inert gas shunt	−0.911*	0.695*
Mean $\dot{V}_A/\dot{Q}$ of perfusion distribution	0.357*	−0.619*
Log standard deviation of perfusion distribution	−0.264	0.263
Inert gas deadspace	−0.284	0.265
Oxygen consumption, indexed to body weight	0.432*	—
Carbon dioxide consumption, indexed to body weight	—	0.169
Core temperature	−0.176	0.307
Mixed venous oxygen tension	−0.181	—

Pao_2, arterial oxygen tension; $Paco_2$, arterial carbon dioxide tension; $\dot{V}_A/\dot{Q}$, ventilation:perfusion ratio.
*$p < 0.001$ for two-tailed test.

trend, at no single time did the $\dot{V}O_2$ differences exceed the adjusted critical alpha, although MAb had no apparent effect on $\dot{V}CO_2$. Similarly, despite efforts to minimize thermal variation, the core temperature profile in MAb recipients was distinctly lower (≈ 0.5° C) than observed in controls, though none of the individual contrasts reached significance. No apparent extrinsic factors accounted for the differences in core temperature profiles; topical hypothermia was not used; ambient temperatures were equivalent (20.4 ± 1.0° C), and operative times were comparable. Neither $\dot{V}CO_2$ nor core temperature overtly influenced $Paco_2$ (Table III). Although PaO_2 and $\dot{V}O_2$ ($r_s = -0.432$) were inversely associated and $\dot{V}O_2$ and core temperature were related ($r_s = 0.501$), no demonstrable dependence of PaO_2 on core temperature was noted. Furthermore, because mixed venous oxygen tension tended to increase during reperfusion (results not shown) and was not associated with PaO_2, arterial oxygenation was unlikely to have been influenced by the increase in $\dot{V}O_2$.[28] Finally, although some component of the $\dot{V}O_2$ difference can be related to variation in core temperature, the similarity in $\dot{V}CO_2$ profiles is not explained, suggesting MAb asserted an independent effect on oxygen utilization.

Left lung compliance was equivalent in control and in MAb donors (Figure 4). Compliance declined linearly with time after reperfusion, and administration of MAb had no effect on this trend. Although no demonstrable effect was seen of MAb on lung compliance, postmortem gravimetric lung water was significantly less in MAb recipient lungs (5.78 ± 1.01 ml/kg) than in untreated control lungs (8.02 ± 1.90 ml/kg), $p = 0.014$, $\eta^2 = 0.383$. As a basis for comparison, lung water in normal dogs is substantially lower than in either of the current study groups (3.39 ± 0.53 ml/kg, n = 10).

With the right lung excluded, pulmonary vascular resistance was abnormal in donors (control, 481 ± 82 dynes · sec · cm^{-5}; MAb, 435 ± 140 dynes · sec · cm^{-5}) (Figure 4). Pulmonary vascular resistance doubled within the initial 30 minutes of reperfusion (control, 908 ± 242 dynes · sec · cm^{-5}; MAb, 1019 ± 328 dynes · sec · cm^{-5}) but was essentially constant after that time. The pulmonary vascular resistance profile was unaltered by MAb. Despite the change in pulmonary vascular resistance, no evidence existed for global reflow limitation, because cardiac output generally increased during reperfusion (results not shown). Both the increase in $\dot{V}D/\dot{V}E$ and the reduction in mean $\dot{Q}$ imply considerable regional variability in perfusion, however.

DISCUSSION

Excluding PaO_2, $PaCO_2$, and pHa as dependent parameters, MAb administration influenced both pulmonary ($\dot{Q}S/\dot{Q}T$, lung water) and systemic (core temperature, $\dot{V}O_2$) manifestations of reperfusion injury. These data thus support our hypothesis that the development of lung reperfusion injury is modulated by an adhesion-dependent mechanism and provide further confirmation of the role of activated leukocytes and their soluble products in the pathophysiology of this disorder.[16,17,29-31] The protection afforded by MAb was only partial, however, and the complete absence of MAb effect on several other indexes (mean $\dot{Q}$, std dev $\dot{Q}$, $\dot{V}D/\dot{V}E$, $\dot{V}CO_2$, compliance, and pulmonary vascular resistance) that were significantly altered very early during reperfusion implies that the propagation of injury involves both CD18 dependent and independent pathways.

One alternate explanation for the beneficial effects of MAb may have been a reduction in

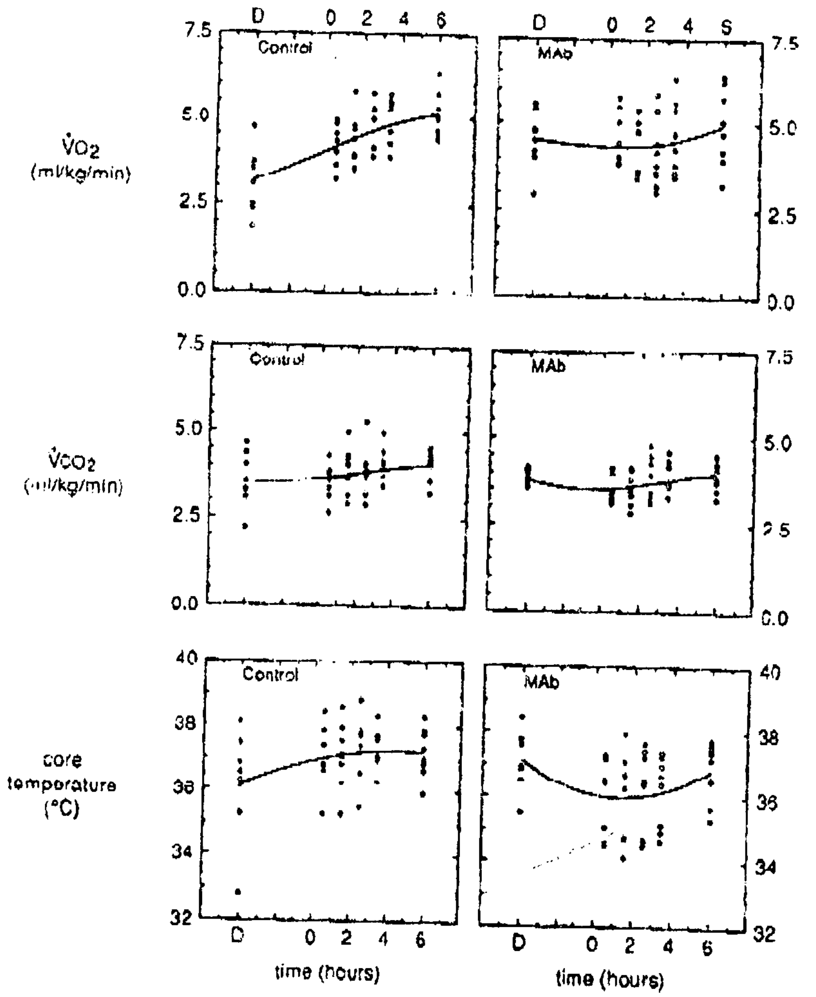

FIGURE 3 Oxygen consumption ($\dot{V}O_2$), carbon dioxide elimination ($\dot{V}CO_2$), and core temperature in controls (left) and R15.7 (MAb)-treated recipients (right) are displayed on the ordinates. Time is displayed on the abscissae. Smoothing as described in Figure 1. The differences in $\dot{V}O_2$ ($p = 0.001$, $\eta^2 = 0.261$) and core temperature ($p = 0.041$, $\eta^2 = 0.230$) profiles suggest MAb administration may have attenuated the release of endogenous pyrogens during the development of an inflammatory process initiated by reperfusion.

circulating leukocyte number as an indirect consequence of MAb binding, without explicit inhibition of β_2 integrin–mediated adhesion. When the systemic effects of an identical dose of R15.7 were studied in a canine myocardial reperfusion model, both the absolute number of neutrophils and the total leukocyte count were reduced by 40% over a 3-hour interval after administration of MAb, even before the initiation of ischemia.[32] Myocardial leukocyte accumulation after ischemia was reduced in MAb recipients, and this effect was shown to be most pronounced during the initial hour of reperfusion. These same investigators found neutrophil CD11b/CD18 expression in cardiac lymph is up-regulated within this interval and have shown a soluble component in cardiac lymph that stimulates neutrophils from normal dogs to up-regulate CD11b/CD18 and develop a bipolar morphology consistent with chemotactic stimulation.[33] Because clearance of ^{99m}Tc-labeled leukocytes from the systemic circulation during reperfusion was reduced in MAb recipients, it is highly probable that interference with adhesion was the specific cause for the reduced inflammatory infiltrate. Nevertheless, substantial tissue damage can occur despite profound (>98%) leukocyte depletion, and until an unequiv-

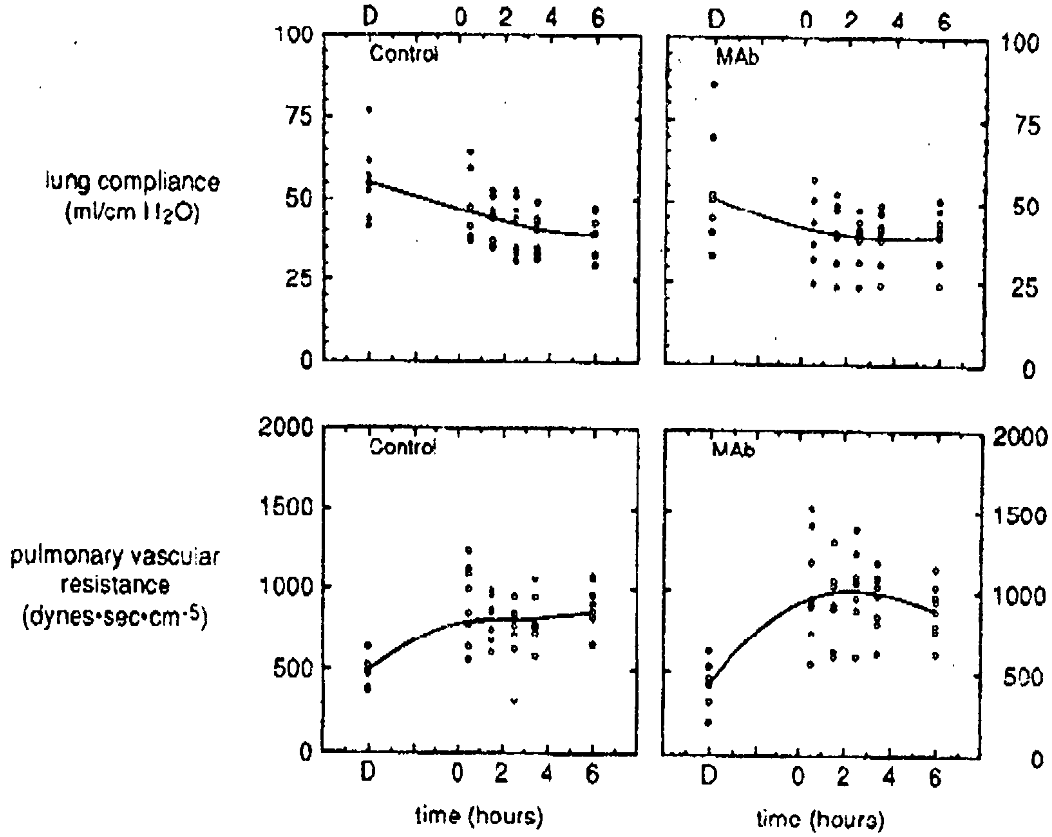

FIGURE 4 Lung compliance and pulmonary vascular resistance in controls (left) and R15.7 (MAb)-treated recipients (right) are displayed on the ordinates. Time is displayed on the abscissae. Smoothing as described in Figure 1. MAb administration did not alter the reduction in pulmonary compliance or the increase in pulmonary vascular resistance that are characteristic of lung reperfusion injury.

ocal threshold number critical to the development of injury is defined, the nonspecific effects of MAb cannot be completely discounted.[34] Although administration of an isotype-matched control MAb directed against an adhesion-independent leukocyte determinant may have been a useful stratagem in resolving this issue, the prospect that inadvertent leukocyte activation or immune complex formation promoted lung injury in controls could not have been excluded without substantial additional study and may well have confounded analysis of the data.[35]

When assessing the possible mechanisms for the development of lung injury in MAb recipients, consideration must be given to the simple prospect that an insufficient dose of antibody was provided. In the few subjects we studied, unbound R15.7 was identified in serum up to 1 hour after the onset of reperfusion, indicating that adequate MAb levels were achieved at least during the initial hour of reperfusion (results not shown). A more comprehensive analysis of R15.7 pharmacokinetics confirms that although the dose used in this study should have been adequate to saturate available receptors, maximum binding may not occur until some time after administration.[32] Interestingly, these workers also noted that a significant fraction of CD18 was not bound despite an excess of MAb, even while the total number of available receptors was down-regulated. Because a similar phenomenon has been observed during the study of the effects of an anti-CD11b MAb (904) in another model of myocardial reperfusion injury, the persistence of free receptors may signal continuous expression in response to ongoing stimulation rather than an inherent discrepancy between *in vitro* and *in vivo* binding affinity.[36]

The observed pattern of physiologic derangement in controls and MAb recipients suggests that lung

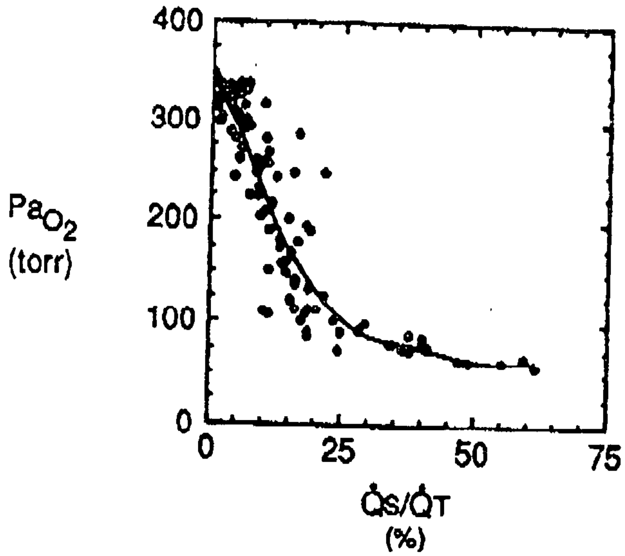

FIGURE 5 The dependence of arterial oxygen tension (PaO_2) on inert gas shunt ($\dot{Q}S/\dot{Q}T$) in lung reperfusion injury. Smoothing as described in Figure 1. Spearman rank correlation = −0.911.

reperfusion injury evolves by at least two concurrent processes, with a rapidly expressed $\dot{V}A/\dot{Q}$ maldistribution (mean $\dot{Q}$, std dev $\dot{Q}$, $\dot{V}D/\dot{V}E$, compliance, and pulmonary vascular resistance) that exerts minimal effect on arterial oxygenation, and a β_2 integrin–mediated component characterized by the progressive loss of endothelial integrity ($\dot{Q}S/\dot{Q}T$, lung water), augmented release of pyrogenic cytokines (core temperature), increased oxidative metabolism ($\dot{V}O_2$), and deteriorating respiratory gas exchange.[37-39] The parallel evolution is in accord with several reports that the preliminary localization of unstimulated leukocytes at sites of inflammation may be dependent on adhesion to stimulated endothelial cells (IL-1, histamine, platelet-activating factor, thrombin, or C5b-9 complex) expressing P-selectin or inducing E-selectin.[40-44] In complementary fashion, constitutively expressed L-selectin mediates leukocyte adherence to cytokine (IL-1, tumor necrosis factor)-stimulated endothelium under conditions of increased flow and high shear and is subsequently shed during neutrophil activation, while CD11b/CD18 expression is synchronously up-regulated.[45-49] Although considerable overlap occurs in the biologic activity of the selectins and β_2 integrins, in aggregate these data would appear to indicate that the rapid evolution of the leukocyte-dependent vascular and airway response in lung reperfusion injury may be selectin mediated, although the development of high-permeability edema and reduced oxygenation are the cumulative result of an integrin and ICAM-facilitated sequence of migration, degranulation, and respiratory burst in response to the iterative elaboration of cytokines, reactive oxygen species, and arachidonate derivatives by sequestered leukocytes and damaged endothelium.[35,50-56] Independent of the precise molecular and cellular processes that alter capillary permeability, the development of arterial hypoxemia in lung reperfusion injury is an unequivocal function of $\dot{Q}S/\dot{Q}T$ (Figure 5).

This work was designed as a preliminary exploration of the physiologic consequence of interfering with leukocyte adhesion during the development of lung reperfusion injury, and although an unequivocal MAb effect was shown, these data provide few definitive answers and suggest several additional areas of investigation. Specifically, the extent to which donor and recipient β_2 integrin expression and avidity are up-regulated by hyperoxic mechanical ventilation and operative trauma before reperfusion was not examined but may well be relevant to the nature and timing of efforts aimed at constraining the effects of activated leukocytes during reperfusion. These same stimuli might well induce pulmonary ICAM-1 expression and compound the ischemic insult. Although hypothermic storage should inhibit de novo ICAM-1 synthesis, endogenous substrate may well be sufficient to permit ICAM-1 up-regulation even during ischemic re-

warming, priming the lung for injury at the onset of reperfusion. Similarly, the duration of the inhibitory effect of MAb is, as yet, undefined, nor is it known whether the risk of perioperative infection would be increased, nullifying the benefits of improved post-reperfusion function

We conclude that inhibition of β_2 integrin–mediated function alters the evolution of abnormal respiratory gas exchange by lungs subjected to hypothermic preservation and reperfusion. The primary effect of MAb administration was to reduce the rate at which $\dot{Q}S/\dot{Q}T$ developed, secondarily limiting the maximum level of $\dot{Q}S/\dot{Q}T$ and, ultimately, restricting the deterioration in arterial oxygen and carbon dioxide tension characteristic of reperfusion injury. Equally important, administration of MAb had no detectable adverse consequences, as assessed by any of the parameters we measured. Although not experimentally verified, the difference in core temperature profiles implies that the release of endogenous pyrogens may have been limited by inhibiting CD18-dependent function, but no evidence exists that the related increase in $\dot{V}O_2$ influenced PaO_2. Finally, these data indicate that interference with β_2 integrin–mediated function curtails reperfusion injury at a relatively slowly developing phase in the evolution of the acute inflammatory process and that additional work to characterize the role of integrin-dependent and selectin-dependent mechanisms in reperfusion injury is warranted.

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SCIENTIFIC SESSIONS DISCUSSION

Craig Smith: I think this is very elegant physiology. On a conceptual level it is very similar to leukocyte depletion, which has been well studied by Dr. Baumgartner and his colleagues at Johns Hopkins and by others. Can you tell us how this compares to the results obtained with leukocyte depletion? Would you expect it to be additive or in some way to be better or worse than leukocyte depletion?

David P. Kapelanski: I think it would have different application. There are other mechanisms of leukocyte activation and adherence besides CD18. We think the advantage this may have over leukocyte filtration is that antibody is present for only a brief period of time in circulation and may be sufficient to limit reperfusion injury without potentially incurring an increased risk of infection. What we like about this method, as well as leukocyte depletion by filtration, is the possibility that by limiting reperfusion injury and release of antigens to the systemic circulation you might limit the incidence of early rejection episodes. There are data that indicate specific tolerance can be induced in rodents using a very similar approach. We would like the opportunity to do a head to head comparison with leukocyte depletion in a single model. We have, thus far, been unable to gain either the filters or sufficient antibody to do this.

Richard Novick: I am a bit perturbed by the relatively poor PO_2s in the control groups, in that after 4 hours of ischemia and 6 hours of reperfusion the PO_2-FIO_2 ratio was only 200. Perhaps you could explain why the gas exchanges were suboptimal in control animals after only 4 hours of ischemia.

Have you used CD18 blockade during longer intervals of ischemia to try to exhibit more prominent differences among groups?

David P. Kapelanski: Let me answer the second question first. We have not had access to enough antibody in purified form to do any other studies as yet. We are working to acquire more. In the second instance, I would dispute the contention that arterial oxygenation was poor. These were open chest animals, essentially without positive end-expiratory pressure. No additional maneuvers were used to influence gas exchange. Furthermore, with the right lung excluded, there is an obligate increase in pulmonary pressure that would tend to augment any injury that occurred. I think these are reasonable data in dogs, and I am not at all embarrassed to show them.

Paul Corris: The theory is fine. You obviously had the opportunity to look at either lavage or histology and quantify the neutrophil influx into the lung. Have you actually done that, and is there a difference?

David P. Kapelanski: No. In point of fact, we have not. We know of data from the Baylor group using a myocardial reperfusion injury model in dogs and the identical antibody demonstrating that neutrophil influx is reduced significantly. You are right that theory is fine; and interestingly enough, newer data would suggest that events are somewhat different than I portrayed and that the effect of antibody may be in limiting neutrophil transendothelial migration, rather than endothelial adherence. There is another class of adhesion molecules we will hear about later on in the paper session, called the selectins, that appear to be more important under conditions of high shear in capturing the leukocytes and holding them adjacent to the endothelial membrane, while CD18 predominately influences transendothelial migration.

Survival in Lung Reperfusion Injury Is Improved by an Antibody that Binds and Inhibits L- and E-Selectin

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The selectins are a three-member family of leukocyte, platelet, and endothelial cell adhesion proteins that mediate leukocyte traffic into normal and inflamed tissues. P-selectin is expressed by endothelial cells and platelets, E-selectin by endothelial cells, and L-selectin by circulating leukocytes. To determine if selectin-mediated leukocyte adhesion influences the development of lung reperfusion injury, we studied hemodynamics and respiratory and inert gas exchange in sheep subjected to 3-hour in situ left lung ischemia followed by 6-hour left lung reperfusion with the right lung excluded. Ten minutes before reperfusion, eight animals received EL-246 (1 mg/kg intravenously), a novel antihuman selectin antibody that recognizes and blocks both L- and E-selectin and cross-reacts in sheep. Eight control animals with ischemia received no treatment, whereas three received an isotype-matched antihuman L-selectin antibody that does not cross-react in sheep (DREG-56, 1 mg/kg intravenously). Eight sham control sheep underwent an identical operative procedure but were never subjected to ischemia. Volume-cycled, pressure-limited (20 cm H_2O) mechanical ventilation was consistent in all animals throughout the experiment. Six-hour survival in EL-246 recipients (100%) was significantly higher than in either ischemic control sheep (37.5%) or DREG-56 recipients (33.3%), but gravimetric lung water was equivalent in EL-246 recipients (5.9 ± 1.7 ml/kg), ischemic control sheep (8.3 ± 3.0 ml/kg), and DREG-56 recipients (9.1 ± 2.6 ml/kg). Although inert gas shunt at ½ hour of reperfusion was no different when contrasted in EL-246 recipients (15% ± 8%), ischemic control sheep (30% ± 25%), and DREG-56 recipients (35% ± 21%), shunts in EL-246 recipients resolved (4% ± 4%) within the 6-hour study period and were associated with a concomitant improvement in respiratory gas exchange. Peripheral blood neutrophil counts increased after both EL-246 and DREG-56 administration, suggesting that the beneficial effect of EL-246 was not incurred by leukocyte depletion. We conclude that mechanisms other than activated neutrophils may account for the initial deterioration of respiratory gas exchange in lung reperfusion injury and inhibition of selectin function improves survival by preventing leukocyte-mediated amplification of this early process. J HEART LUNG TRANSPLANT 1994;13:306-18.

The biochemical signals and biophysical processes required for the precise orchestration of leukocyte traffic in acute inflammation have recently been subject to increasing scrutiny. Although the inciting stimuli are diverse, enrollment of individual leukocytes in the sequence that begins with margination under high shear conditions and culminates in extravascular migration is contingent on a reversible, low-affinity adhesive interaction with activated endothelium.[1-5] In large part this preliminary interaction is mediated by a family of surface glycoprotein monomers collectively termed the selectins.[6-9]

The archetype of the selectin family comprises an aminoterminal lectinlike domain, for recognition of and calcium-dependent binding to oligosaccharides, an epidermal growth factor–like domain that may further regulate ligand specificity, two or more

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consensus repeat domains homologous to the complement regulatory proteins, a brief transmembrane domain, and a short cytoplasmic tail.[10,11] Structural homology notwithstanding, each member of the selectin family is functionally discrete, with a unique cellular distribution and a distinct pattern and tempo of expression ranging from the hours required for transcriptional activation of endothelial E-selectin to minutes for translocation of endothelial and platelet P-selectin from cytoplasmic storage granules to the surface membrane. In contrast, constitutively expressed leukocyte L-selectin is rapidly down-regulated during activation. Because β_2-integrin expression is synchronously upregulated, L-selectin shedding may be a prerequisite for integrin-mediated firm adhesion and subsequent transendothelial migration by activated leukocytes.[12-19]

Our interpretation of this scheme suggested that it might be possible to alter the evolution of lung reperfusion injury by restricting selectin-mediated function. We tested this hypothesis by administering a selectin-blocking antibody immediately before the onset of reperfusion in sheep subjected to 3 hours of normothermic lung ischemia. The antiselectin antibody (EL-246) used in these studies was originally shown to recognize and block both human L- and E-selectin.[20] EL-246 cross-reacts in a number of different animals, but its effectiveness in blocking selectin function in vivo has not been tested previously.

MATERIAL AND METHODS

General Methods

General anesthesia was induced in fasting, nonpregnant ewes (weight 24 to 30 kg) with thiopental sodium (500 mg intravenously). Cimetidine hydrochloride (300 mg intravenously) and diphenhydramine hydrochloride (25 mg intravenously) were administered to minimize the potential effects of histamine release associated with administration of anesthetic and muscle relaxants.[21-27] After oral intubation and evacuation of the rumen, a cervical tracheostomy was performed, and volume-cycled, pressure-limited ventilation was provided with a 10.0 mm cuffed tube (ventilator rate = 13 breaths/min, inspired O_2 fraction = 0.53, inspiratory/expiratory ratio = 1:2, inspiratory pressure limit = 20 cm H_2O, maximum tidal volume = 700 ml, and positive end-expiratory pressure = 5.0 cm H_2O). These ventilator settings were not altered during the experiment. A deep plane of surgical anesthesia and complete paralysis were maintained throughout the experiment by continuous administration of both fentanyl citrate (1.5 mg/hr intravenously) and pancuronium bromide (3.0 mg/hr intravenously). The methods used for physiologic support and monitoring, including determination of continuous ventilation/perfusion distributions, have been described in detail previously.[28]

Experimental Protocol

The left lung was exposed in prone subjects by excision of several adjacent ribs, including the intercostal muscles and neurovascular structures. Minimizing manipulation of the left lung, potential systemic–to–pulmonary artery collaterals were interrupted by division of the pulmonary ligaments and hilar skeletonization, which included division of the bronchial arteries. After completing the intrathoracic dissection, a 30-minute recovery period was provided. At the conclusion of that interval, six animals with arterial O_2 tension (PaO_2) less than 200 mm Hg or arterial CO_2 tension ($PaCO_2$) greater than 45 mm Hg were excluded from further study. Baseline physiologic data during bilateral lung ventilation and perfusion were then recorded in all remaining subjects. After systemic heparinization (300 units/kg intravenously), a 3-hour period of normothermic left lung ischemia was initiated in 19 animals by occlusion of the left main pulmonary artery. In eight additional sheep, both lungs were ventilated and perfused during this 3-hour interval.

Ten minutes before reperfusion of the left lung, eight subjects received a bolus infusion (1.0 mg/kg intravenously, in 25 ml 0.9% saline solution) of EL-246, an immunoglobulin G_1 murine monoclonal antibody that binds an epitope in the short consensus repeats of L- and E-selectin from humans and several other species, including sheep.[20] Three subjects with ischemia received a bolus infusion (1.0 mg/kg intravenously in 25 ml 0.9% saline solution) of DREG-56, an identical subtype antibody that binds a different human L-selectin epitope not present in sheep. Both EL-246 and DREG-56 exhibit approximately equivalent in vitro inhibition of L-selectin–mediated human leukocyte adhesion.[29,30] Eight ischemic control sheep and eight sham control sheep received no treatment. Ten minutes after the onset of reperfusion, during which both lungs were ventilated and perfused, the right pulmonary artery was ligated. Simultaneously, the tip of the endotracheal tube was advanced beyond the orifice of the tracheal bronchus and the right main bronchus was clamped at end expiration. The right lung of sham control sheep was similarly excluded 3 hours after the

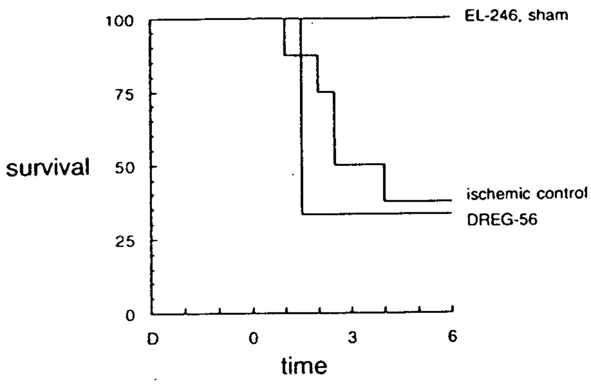

FIGURE 1 Group survival (percent) as a function of time (hours), beginning with initial dual lung determination *(D)*. Zero on abscissa marks onset of reperfusion in animals subjected to left lung ischemia. Survival of EL-246 recipients was significantly higher than that of either ischemic controls ($p = 0.011$) or DREG-56 recipients ($p = 0.015$), whereas there was no difference when survival of ischemic controls and DREG-56 recipients was compared ($p = 0.464$).

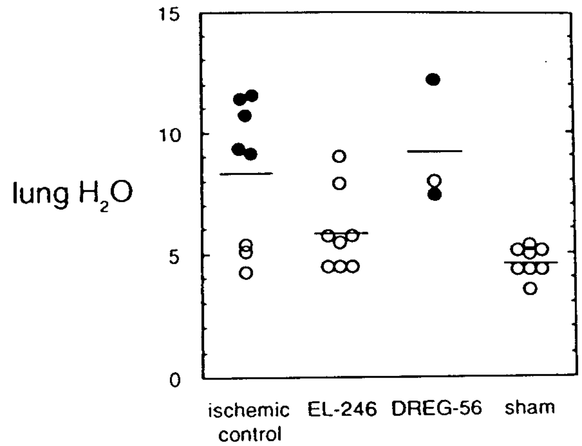

FIGURE 2 Individual gravimetric lung water (milliliters per kilogram) determinations by group. *Open circles* identify subjects surviving planned observation period; *filled circles* indicate nonsurvivors. Group means are identified by *horizontal bar*. An increase in lung water was a feature generally characteristic of nonsurvivors. The trend suggesting only limited increase in lung water in EL-246 recipients was not significant ($p = 0.085$; power $= 0.485$).

accumulation of baseline data. Physiologic parameters were recorded in all subjects 3½, 4½, 5½, 6½, and 9 hours after baseline determinations (½, 1½, 2½, 3½, and 6 hours after the onset of reperfusion). Serum titers of EL-246 and DREG-56 were determined at ½, 1½, 2½, 3½, and 6 hours of reperfusion, according to previously described methods.[20] Peripheral blood leukocyte counts and differentials were assessed in EL-246 and DREG-56 recipients at identical times. Sodium bicarbonate was administered as necessary to correct metabolic acidosis. Airway secretions were aspirated as necessary to maintain the airway, but aspiration always preceded physiologic measurements by a minimum of 20 minutes. Heating blankets and warming lamps were used to prevent variation in core temperature. No additional resuscitative or supportive measures were used.

Euthanasia was performed at the end of each experiment by KCl overdose in completely anesthetized survivors. Death was verified before autopsy, at which time the left lung was excised and gravimetric lung water determined. All animals received humane care in compliance with the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, and the "Guide for the Care and Use of Laboratory Animals," prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Data Analysis

All summary data are tabulated as the mean ± standard deviation. Gravimetric lung water in subjects with ischemia was compared by a single-factor (group) analysis of variance (ANOVA). The planned analysis of serial data from animals with ischemia was a two-factor (group × time) repeated-measures ANOVA with the use of all six data collection periods. Although the number of censored observations in two groups (control animals with ischemia and DREG-56 recipients) invalidated this evaluation, a similar analysis during the interval in which no deaths occurred (baseline through 30 minutes of reperfusion) was not limited by censoring. A repeated-measures analysis of covariance was used in two instances to adjust for the known dependence of a variable on a second, independently measured parameter. Data from the sham group were accumulated primarily to assess the physiologic response to operative trauma and prolonged single-lung support in sheep. To provide additional insight into the effects of EL-246 administration, a second main-effects analysis with the use of all six data collection points was used to contrast EL-246 recipients and sham control animals, as described previously. The Huyn-Feldt correction was used in all repeated-measures analyses.[31] In all instances, the critical α

for main effects was 0.05. Power and treatment magnitude (η^2) were calculated for all ANOVA and analysis of covariance group effects.[32] Scheffe's adjusted critical F was used in all post hoc comparisons of serially accumulated physiologic data. Survival was compared by the actuarial method, with a Bonferroni adjusted α of $0.05 \div 3$ contrasts $= 0.017$ to preserve the overall experimental α of 0.05.[31,33] All statistical analyses were accomplished with SPSS software routines (SPSS Inc., Chicago, Ill.).

RESULTS

Only three of eight control sheep with ischemia survived the experimental observation period (Figure 1). Administration of DREG-56 provided no advantage because only one of three sheep survived the planned duration. Most deaths in both of these groups occurred within the initial 3 hours of the onset of reperfusion, and, in each instance, death could be attributed to a combination of respiratory failure and circulatory collapse. Survival of EL-246 recipients was uniform during the study interval and significantly exceeded that achieved by either control animals with ischemia or DREG-56 recipients.

Analysis of postmortem gravimetric lung water and serially determined physiologic parameters (Figures 2 through 8; Table I) provided only limited information about specific mechanisms that might have contributed to the improvement in survival achieved by EL-246 recipients. A precipitous deterioration in respiratory gas exchange was evident in the majority of animals after ischemia within the initial 30 minutes of reperfusion, and the combination of hypoxemia, severe hypercapnia, and marked respiratory acidosis usually portended early death. Hypercapnia and acidosis were initially less pronounced in survivors, and although a residual component of CO_2 retention often persisted, the majority of animals tended to improve during the later hours of the experiment. Similarly, the preliminary reduction in PaO_2, although widespread, was less severe in experimental survivors, and by 6 hours of reperfusion the mean PaO_2 in EL-246 recipients averaged 224 ± 56 mm Hg, only 17% below the baseline determination of 272 ± 23 mm Hg. Inert gas shunt ($\dot{Q}_S/\dot{Q}_T$), the primary determinant of abnormal respiratory gas exchange in lung reperfusion injury, increased between fivefold and tenfold during the initial 30 minutes of reperfusion, although there was considerable variation within groups and, by this criterion, minimal objective evidence of injury in some animals.[28] As with PaO_2 and $PaCO_2$, there was a tendency toward renormalization of $\dot{Q}_S/\dot{Q}_T$ in surviving animals. This delayed recovery was particularly prominent in EL-246 recipients because, with one exception, $\dot{Q}_S/\dot{Q}_T$ measured in each EL-246 recipient at the conclusion of the study was within 3% of the value initially determined for that same subject before ischemia. The overall trends in minute ventilation ($\dot{V}_E$), pulmonary vascular resistance (PVR), and inert gas dead space ($\dot{V}_D/\dot{V}_E$) in subjects with ischemia were comparable, with moderate deterioration evident at the initial postreperfusion measurement and minimal subsequent change in surviving animals. A statistical summary of the comparisons of PaO_2, $PaCO_2$, $\dot{Q}_S/\dot{Q}_T$ (adjusted for cardiac output), $\dot{V}_E$, PVR, and $\dot{V}_D/\dot{V}_E$ (adjusted for $\dot{V}_E$) in animals subjected to ischemia is presented in Table I.[34-36] None of these tests identified differences between control sheep with ischemia, EL-246 recipients, and DREG-56 recipients during the interval concluding at 30 minutes of reperfusion. In several instances the power of the individual tests suggested that experimental design may have been a critical factor in this outcome and an effect of EL-246 might well have been detected by analysis of a numerically larger sample.

From the preceding, a paradox is apparent: administration of EL-246 significantly enhanced survival after ischemia without overtly altering the initial expression of lung reperfusion injury. Because of this anomalous result, with an evident trend toward improvement of serially assessed parameters in EL-246-treated subjects, a second main-effects analysis was undertaken, comparing EL-246 recipients with sham-treated control animals during all six data periods. The results of this analysis are summarized in Table II. Four criteria (PaO_2, $PaCO_2$, $\dot{Q}_S/\dot{Q}_T$, and PVR) suggested that left lung function deteriorated significantly after ischemia despite administration of EL-246, whereas two parameters ($\dot{V}_E$ and $\dot{V}_D/\dot{V}_E$) failed to identify any consequence of ischemia and reperfusion. When an analysis of simple effects was used to compare significant trends further (PaO_2, $PaCO_2$, $\dot{Q}_S/\dot{Q}_T$, and PVR), a consistent pattern emerged: both groups were equivalent at the outset of the experiment and, with the exception of a persistent elevation of PVR in EL-246 recipients, both groups were again indistinguishable by 6 hours of reperfusion.

Although the intended effect of EL-246 was inhibition of L- and E-selectin–mediated adhesion, an equivalent benefit might have accrued if administration of antibody resulted in leukopenia. Neutrophil number, assessed in all three DREG-56

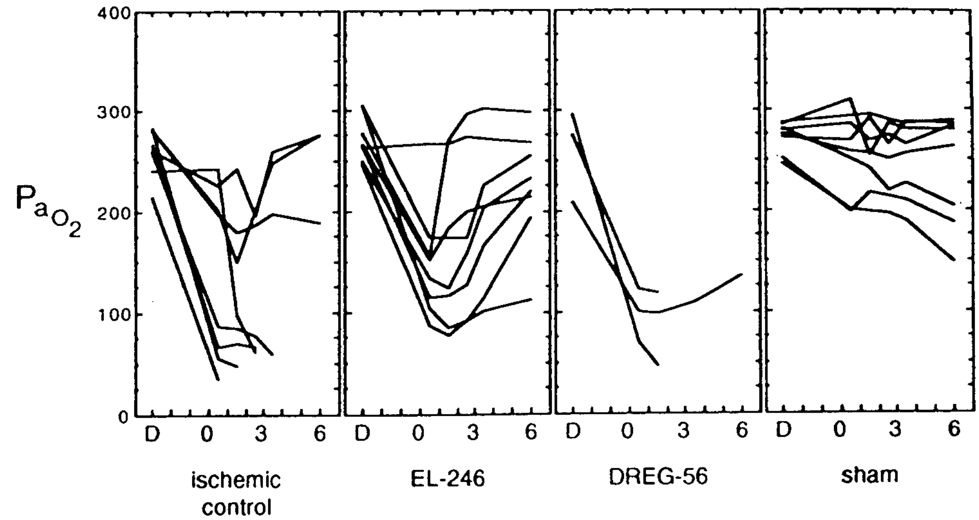

FIGURE 3 Pa_{O_2} (mm Hg) determinations for each subject are stratified by group, with experimental time indicated on abscissa. Initial Pa_{O_2} reduction was same among three groups with ischemia, but EL-246 recipients were not different from sham controls by 6 hours of reperfusion.

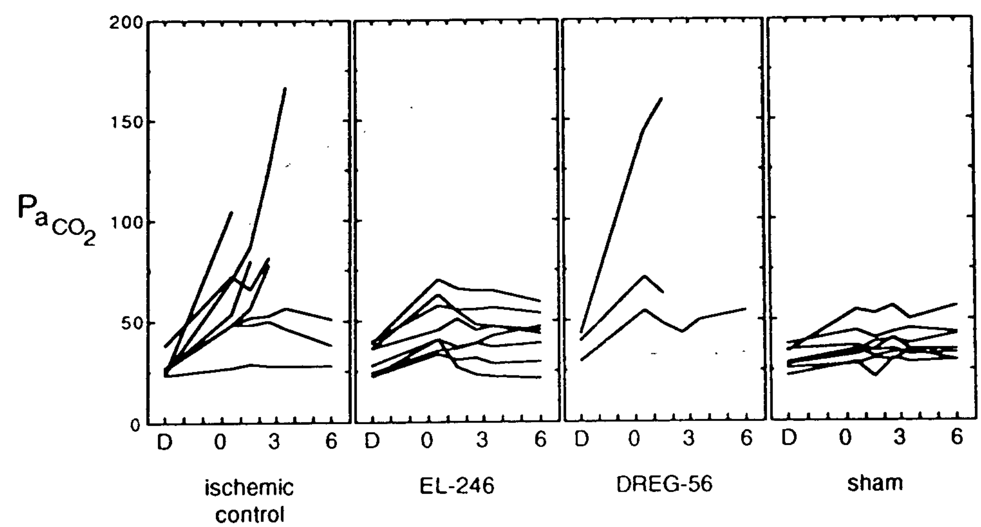

FIGURE 4 Pa_{CO_2} (mm Hg) for each subject. Severe hypercapnia and respiratory acidosis developed rapidly in nonsurviving subjects, but early Pa_{CO_2} reduction was identical when three groups with ischemia were compared. Pa_{CO_2} trends in EL-246 recipients and sham controls were not parallel, but differences at individual times were not identified by post hoc comparison.

recipients and seven EL-246 recipients (Figure 9), tended to increase in EL-246 recipients throughout the experiment, and although, the initial pattern in DREG-56 recipients appeared comparable, the early deaths of two sheep in this group limited the opportunity to characterize the trend further. When analyzed during the three sampling points for which complete data were available (baseline through 90 minutes of reperfusion), no differences in neutrophil count were identified. A similar conclusion was reached after analysis of total leukocyte number (not shown).

The results of the serial assessment of serum EL-246 titer are illustrated in Figure 10. These data

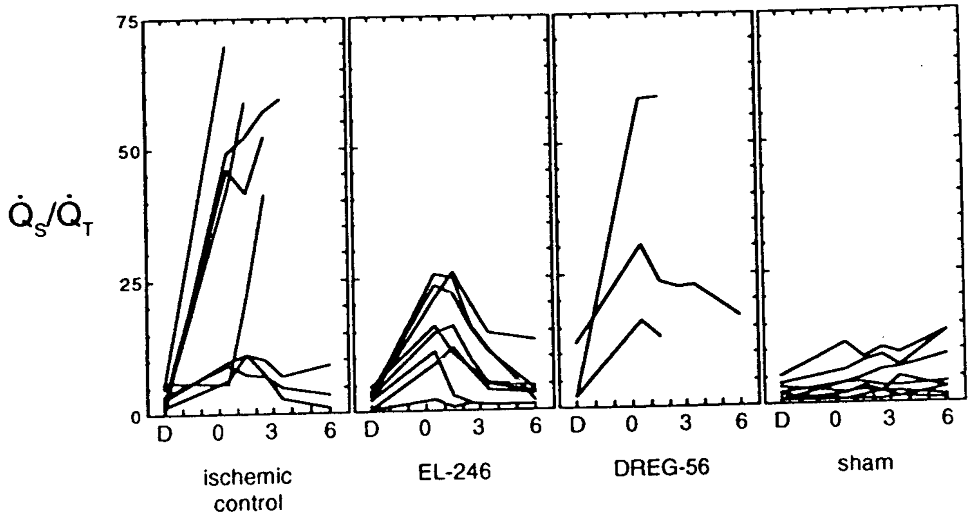

FIGURE 5 $\dot{Q}_S/\dot{Q}_T$ (percent) for each subject. Initial increase in $\dot{Q}_S/\dot{Q}_T$ was comparable in each group with ischemia. EL-246 recipients were distinguished by virtually complete resolution of $\dot{Q}_S/\dot{Q}_T$ within 6-hour observation period and were not different from sham controls by termination of experiment.

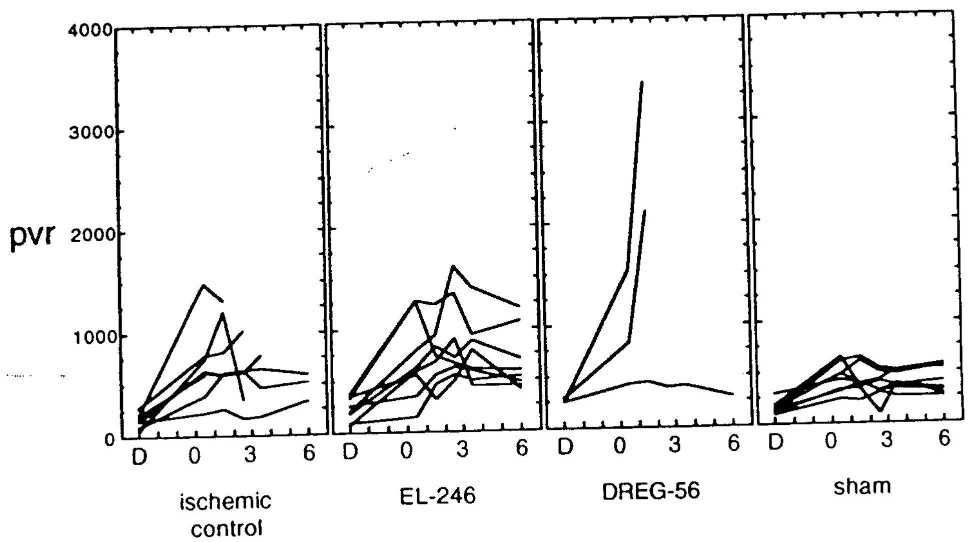

FIGURE 6 Pulmonary vascular resistance (*PVR*) (dynes · sec · cm^{-5}) for each subject. There were no differences between groups with ischemia when initial increase in PVR was tested. PVR in EL-246 recipients was persistently higher than in sham controls after initial 30 minutes of reperfusion.

confirm that the administered dose of antibody was sufficient to maintain high levels of unbound EL-246 throughout the 6-hour reperfusion period and further suggest that the apparent failure of this antibody to influence the initial evolution of lung reperfusion injury was related to other, unstudied mechanisms.

DISCUSSION

In a previous study of canine lungs transplanted after 4-hour hypothermic preservation, we described the results obtained when an antibody (R15.7) directed against an epitope of the common subunit (CD18) of the β_2-integrins was administered to lung recipients immediately before graft reper-

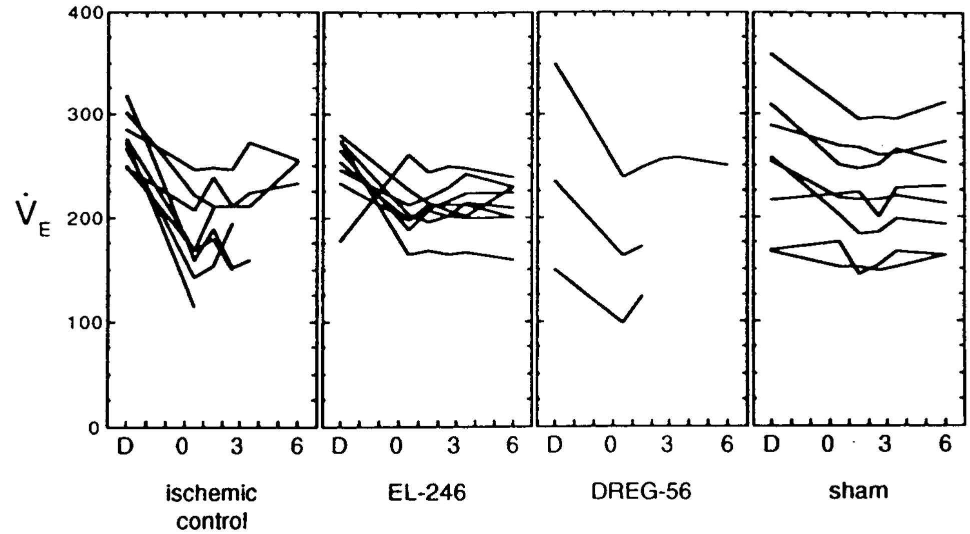

FIGURE 7 $\dot{V}_E$ (milliliters per kilogram per minute at body temperature and ambient pressure and saturated with water vapor) for each subject. Initial reduction in $\dot{V}_E$, an indirect index of lung compliance, was equivalent in each group with ischemia. Aggregate trends in EL-246 recipients and sham controls were no different, suggesting initial reduction in $\dot{V}_E$ was largely consequence of conversion from dual to single lung ventilation.

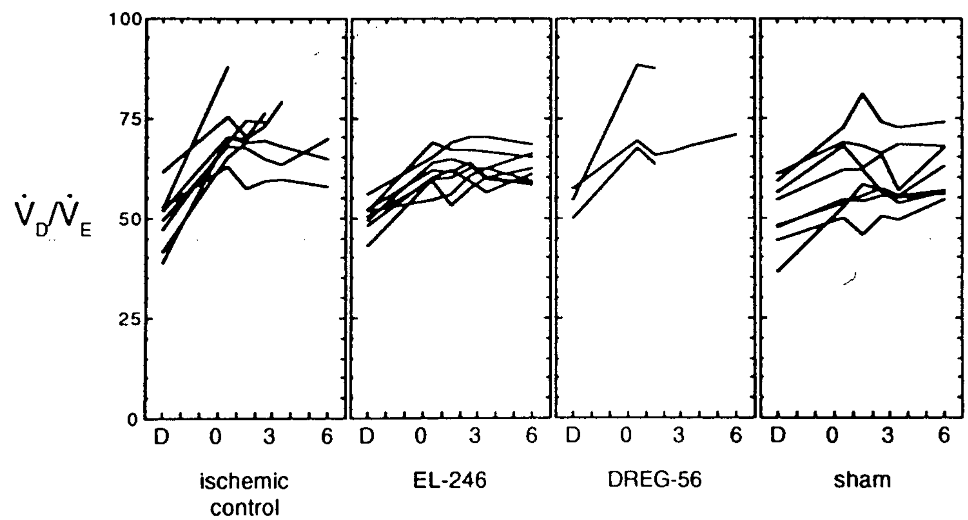

FIGURE 8 $\dot{V}_D/\dot{V}_E$ (percent) for each subject. When adjusted for reduction in $\dot{V}_E$ that occurred during transition from dual to single lung ventilation, $\dot{V}_D/\dot{V}_E$ was comparable in all groups. Bohr dead space was approximately 8% to 10% higher than $\dot{V}_D/\dot{V}_E$ at each determination, but fundamental trends were equivalent and outcome of analysis was identical.

fusion.[28] Although the majority of R15.7 recipients had features of acute lung injury, the magnitude of the impairment in both respiratory and inert gas exchange was curtailed by R15.7 treatment. When the evolution of the gas exchange abnormality was analyzed in greater detail, it was evident that the consequences of inhibiting β_2-integrin function were relatively negligible during the early hours of reperfusion. Several mechanisms could have accounted for the delayed onset of antibody effect,

TABLE I Main group effects in the comparison of control animals with ischemia, EL-246 recipients, and DREG-56 recipients

	Within subjects		Between subjects	
	p Value	Power	*p* Value	Power
Pao_2	0.485	0.157	0.676	0.106
$Paco_2$	0.059	0.554	0.082	0.493
$\dot{Q}_S/\dot{Q}_T$	0.306	0.235	0.084	0.486
PVR	0.776	0.086	0.508	0.149
$\dot{V}_E$	0.123	0.412	0.618	0.119
$\dot{V}_D/\dot{V}_E$	0.168	0.350	0.088	0.478

TABLE II Main group effects in the comparison of EL-246 recipients and sham control animals

	Within subjects			Between subjects		
	p Value	Power	η^2	*p* Value	Power	η^2
Pao_2	0.024		0.316	<0.001		0.386
$Paco_2$	0.242	0.205		0.017		0.196
$\dot{Q}_S/\dot{Q}_T$	0.076	0.431		0.001		0.505
PVR	0.007		0.415	0.025		0.179
$\dot{V}_E$	0.670	0.063		0.607	0.157	
$\dot{V}_D/\dot{V}_E$	0.269	0.457		0.776	0.055	

η^2, Treatment magnitude.

including the prospect that the latency accurately reflected the time required to complete some critical preliminary phase of the inflammatory process and reach a state in which inhibition of integrin-dependent activity might be possible.

On the basis of that study and emerging information concerning the sequential participation of the selectins and β_2-integrins in acute inflammation, we hypothesized that the physiologic expression of lung reperfusion injury might be equivalently reduced by restricting selectin-mediated function.[3,5,18,19,37-39] Furthermore, we speculated that the latent period might be eliminated if we successfully intervened at an earlier phase in leukocyte recruitment. Although our original goal was to explore the effects of selectin inhibition in the same canine model, we were unable to identify a blocking antibody that was effective in dogs. We ultimately resorted to the current study design when we were unable to obtain even limited short-term survival of sheep lung recipients. Although several factors may have contributed to acute graft failure, we believe that the primary mechanism was airway hyperactivity associated with rapid rewarming because the transplanted lungs resisted efforts at reinflation and none of the grafts could be ventilated reliably.[40-45] In the end the model we report required an analytic compromise because isolated study of left lung function before ischemia would have necessitated transient ischemia of the right lung and might have prematurely provoked leukocyte activation and bilateral lung injury. Data accumulated at baseline were accumulated primarily to define the overall status of the cardiorespiratory system before ischemia and, in the case of sham control animals, to illustrate the concerted physiologic effects of operative trauma, prolonged anesthesia, and the transition from dual to single lung function without an intervening ischemic period. Although we recognize the inherent flaw in the comparison of data acquired in these two distinct states, we believe that by providing a reference for variation within each subject, use of the baseline data effectively increased the overall sensitivity of the main analysis to the designed groupings.

Specific problems encountered during the execution of this study merit brief additional consideration. As stated previously, six subjects were summarily excluded after preliminary evaluation of dual lung function, before ischemia. In each instance, parenchymal edema, visceral pleural weeping, and impaired respiratory gas exchange were evident.

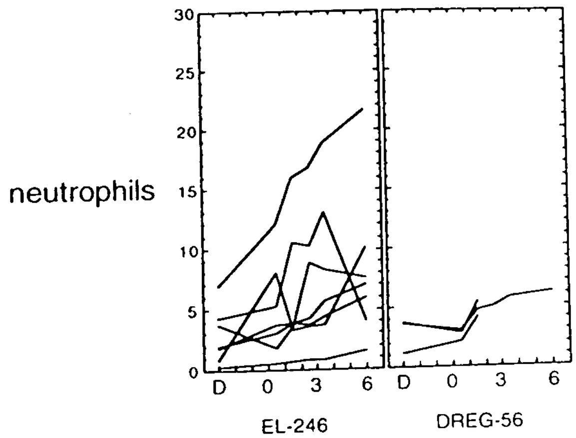

FIGURE 9 Peripheral blood neutrophils (· 10^3 per cubic millimeter) for antibody recipients. Effect of EL-246 on survival was not mediated by reduction in circulating neutrophils (between-subjects effect of group: $p = 0.609$, power = 0.075; within-subjects effect of group: $p = 0.533$, power = 0.142).

These abnormalities may have developed as a consequence of lung overinflation despite incorporation of an inspiratory pressure release in the ventilator circuit. Because one objective of this study was to provoke high-permeability edema, and the characteristic features of overexpansion injury are identical, inclusion of data from these subjects might have biased the outcome of the study in an unpredictable fashion.[46,47] However, even though withdrawal occurred after declaration of group assignment, we believe the outcome of the study was not altered by these early exclusions. In contrast, the high mortality rate in control sheep with ischemia and DREG-56 recipients severely hindered the analysis of physiologic data during reperfusion, because the times available for study were severely restricted. Because our analysis suggests that EL-246 administration minimally influenced the initial expression of lung injury, we have tabulated certain ancillary information for each statistic. Given the striking influence of EL-246 on survival and the virtually complete resolution of injury in treated animals, we believe the factors contributing to this negative conclusion merit proportionately greater scrutiny.

The physiologic data reported herein are consistent with a biphasic model of lung injury during reperfusion. The first phase is evident within minutes of reperfusion and is not altered appreciably by EL-246 because no significant differences in respiratory or inert gas exchange were reliably identified when the three ischemic groups were compared, although some trends were suggested. However, a secondary amplification phase is modified by EL-246, and interference with this later phase not only confers a significant survival advantage but also facilitates resolution of the initial lesion and allows restoration of near-normal respiratory and inert gas exchange. The EL-246 epitope is expressed by both L- and E-selectin, and this antibody effectively blocks the function of both molecules in different in vitro adhesion assays.[20] EL-246 does not recognize P-selectin, which is rapidly (within minutes) mobilized to the surface of endothelial cells and platelets at sites of inflammation.[20,48-53] The recent demonstrations that the development of myocardial reperfusion injury and complement-mediated lung injury are attenuated by a specific anti-P-selectin antibody or an oligosaccharide that interferes with P-selectin binding suggests an analogous role for P-selectin in this model, and this unconstrained activity may contribute to the initial phase of reperfusion injury that was not significantly altered by EL-246 administration.[54,55] The observation that EL-246 preferentially inhibits the delayed phase of reperfusion injury implies that some component of the EL-246 effect may be related to interference with E-selectin, because expression of E-selectin in inflamed tissues is dependent on de novo protein synthesis, and maximal expression requires approximately 2 to 4 hours.[56-59] L-selectin, which is constitutively expressed, also asserts an independent regulatory effect on leukocyte recruitment into certain inflammatory sites.[1,13,16-18] In addition, L-selectin may be important for P- and E-selectin function, because adhesion of neutrophils to P- and E-selectin–transfected COS cells is reduced by an antibody that recognizes L-selectin but not E- or P-selectin.[17,60] Accordingly, interference with L-selectin may have accounted for the partial but statistically insignificant trends identified at the beginning of reperfusion, whereas the significant effects at later time points might reflect the combined inhibition of both E- and L-selectin. Precise definition of the role of individual selectins in this model will require the development of reagents that specifically inhibit sheep P-, L-, and E-selectin.

Although survival and physiologic recovery were clearly associated with EL-246 treatment, either cimetidine or diphenhydramine might have facilitated the resolution of lung injury by separate and independent mechanisms. The cytochrome P-450 system generates hydrogen peroxide and superoxide

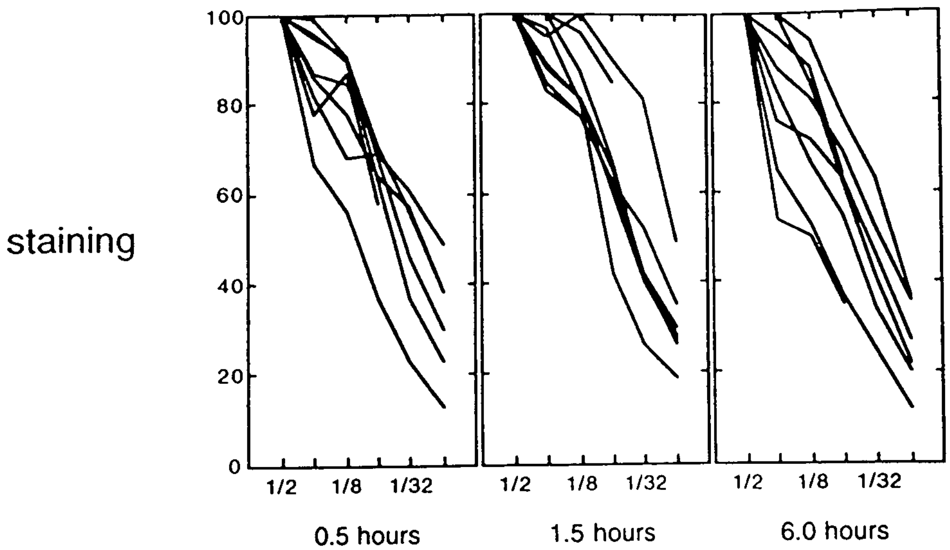

FIGURE 10 Serum EL-246 titers at ½, 1½, and 6 hours of reperfusion were assessed by flow cytometry after incubation of serial twofold dilutions (1:2 through 1:64) with the E-selectin cDNA transfected mouse pre-B-cell lymphoma line L.5. Maximal staining was defined with purified EL-246 and the identical cell line. Initial physiologic deterioration noted during reperfusion occurred despite maintenance of saturating levels of EL-246 throughout experiment. DREG-56 titers were comparable at each period.

anion during normal oxidative metabolism, whereas degradation of the P-450 heme complex as a result of injury may release iron, which can promote lipid peroxidation and catalyze hydroxyl radical production.[61-64] Cytochrome P-450 is widely distributed in the lung and is competitively inhibited by cimetidine but not other H_2-receptor antagonists.[65-69] Recent observations with two different injury models suggest that cimetidine-mediated inhibition of the pulmonary cytochrome P-450 system can dramatically attenuate the development of acute lung injury.[70,71] Because each subject in this study received cimetidine and survival was not overtly influenced, our data suggest that any effect attributable to cimetidine was modest. However, we cannot exclude an auxiliary role in the recovery of lung function in those subjects in whom leukocyte margination, activation, and extravascular migration were altered by EL-246 administration.

The potential modulating effect of diphenhydramine in this model is equally difficult to ascertain. Histamine stimulation provokes a rapid increase in endothelial intracellular calcium, and there is an associated quantitative reduction in F-actin content.[72,73] The normal morphologic pattern of actin filaments and stress fibers is secondarily altered, a finding that coincides with the development of intercellular gaps and a transient, reversible increase in endothelial permeability.[72,74] Furthermore, endothelial surface expression of tissue plasminogen activator, von Willebrand factor, platelet-activating factor, and P-selectin is upregulated within minutes of H_1-receptor stimulation, and each of these effects can be completely blocked by prior treatment with H_1 but not H_2-receptor antagonists.[48-50,73,75] Although mast cell degranulation has been noted in a recent canine study, the role of histamine in the development of lung reperfusion injury is at present ill defined.[76] Thus whether limiting a histamine-stimulated increase in endothelial permeability or by constraining the coordinate expression of platelet-activating factor and P-selectin and thereby reducing leukocyte recruitment and activation, an ancillary role for diphenhydramine in limiting the extent of injury and promoting recovery of lung function cannot be discounted, although survival rates were not improved in this study.[77]

This work was designed to investigate the initial evolution of lung reperfusion injury when preservation was avoided and leukocyte recruitment was impaired, and although an unequivocal physiologic advantage was demonstrated in EL-246 recipients, the potential restriction of both E- and L-selectin activity precludes specific inferences concerning the role of individual members of this family in this

model. Although studies to discriminate the precise activity of each of the selectins are warranted and may ultimately alter clinical practice, even EL-246-treated subjects had significant injury at the outset of reperfusion, suggesting that efforts to decipher the initial endothelial activation signals and interfere with the mechanisms for transduction and propagation of those signals may provide greater benefit.

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Scientific Sessions Discussion

Thomas M. Egan: We, too, are interested in trying to predict which lungs are going to demonstrate poor gas exchange after an ischemic insult and which lungs are not. Some of your control lungs did well, some of them did not. Do you have any feel for what might have predicted their behavior; in other words, could you predict which lungs would have benefited from your intervention?

John B. Steinberg: No, we could not. We set up our experiment so that the initial set of parameters was comparable. We excluded animals that were outside of these confines. Consequently, every animal started out with comparable baseline data. We had no clue as to which animals were going to survive.

Alistair I. Fyfe: An alternative way of inhibiting this process is to compete with the use of exogenous sugars such as galactose or fucose. I wondered if you have tried experiments to see if you can obviate the need for antibodies by using something more physiologic?

John B. Steinberg: No, we have not done that. However, there are studies that demonstrate competitive inhibition of the selectins with the use of exogenous sugars.

William A. Baumgartner: I think you are on the right track, and it was a nice presentation. I think we are all convinced that the white cell plays some role in this. Because your results were not overwhelming as far as showing positivity, did you look to see whether white cells accumulated in the lungs? With myeloperoxidase staining or other techniques, did you determine whether the antibody had an effect on your experiment?

John B. Steinberg: No, we did not look to see whether white cells were incorporated in the lung tissue. However, we did measure the leukocyte counts in the peripheral blood at all set time intervals at which we collected data. The numbers of leukocytes increased throughout the day.